

61-03-00

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Atty Dkt No. 1621.002
2302-1621

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APPLICATION TRANSMITTAL LETTER

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Transmitted herewith for filing is the patent application of Susan BARNETT, Jan ZUR MEGEDE, Indresh SRIVASTAVA, Ying LIAN, Karin HARTOG, Hong LIU, Catherine GREER, Mark SELBY and Christopher WALKER for IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND PRODUCTION OF VIRUS-LIKE PARTICLES, claiming priority to provisional patent applications serial nos. 60/114,495, filed December 31, 1998 and 60/168,471 filed December 1, 1999.

Enclosed are:

121 sheets of drawings.

 A claim for foreign priority under 35 U.S.C. § 119/363 in
 a separate document the declaration.

X A claim for priority under 35 U.S.C. § 119(e)(1) in
 a separate document X the declaration.

 A certified copy of the priority document.

 Verified Statement(s) Claiming Small Entity Status.

X Other: Title page; Sequence Listing on paper (pp.1-62) and on disk; Statement to Support Filing and Submission in Accordance with 37 C.F.R. §§ 1.821-1.825; return receipt postcard.

The declaration of the inventor X is enclosed X unsigned.

The fee has been calculated as follows:

A. Basic Application Fee		\$760.00
B. Total Claims $90 - 20 = 70$	x \$18.00	1260.00
C. Independent Claims $2 - 3 = 0$	x \$78.00	0.00
D. If multiple dependent claims present, add	\$260.00	0.00
E. Total Application Fee (Total of A, B, C, & D)	=	2020.00
F. If verified statement of small entity status is enclosed, reduce Total Application Fee by 50%		0
G. Application Fee Due (E - F)	=	2020.00
H. Assignment Recording Fee of \$40.00 if assignment document is enclosed	\$40.00	NA
I. TOTAL FEE (G + H)		\$2020.00

Respectfully submitted,

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Application for U.S. Letters Patent Entitled

IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND
PRODUCTION OF VIRUS-LIKE PARTICLES

claiming priority to provisional patent applications serial nos. 60/114,495,
filed December 31, 1998 and 60/168,471, filed December 1, 1999

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Attorney Docket No. 1621.002

650227 "EL 457 530 443 US"

IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND
PRODUCTION OF VIRUS-LIKE PARTICLES

CROSS-REFERENCE TO RELATED APPLICATIONS

5 This application is related to provisional patent applications serial nos. 60/114,495, filed December 31, 1998 and 60/168,471, filed December 1, 1999, from which priority is claimed under 35 USC §119(e)(1) and which applications are incorporated herein by reference in their entireties.

TECHNICAL FIELD

10 Synthetic expression cassettes encoding the HIV polypeptides (e.g., Gag-, pol-, prot-, reverse transcriptase, Env- or tat-containing polypeptides) are described, as are uses of the expression cassettes. The present invention relates to the efficient expression of HIV polypeptides in a variety of cell types. Further, the invention provides methods of producing Virus-Like Particles (VLPs), as well as, uses of the VLPs and high level
15 expression of oligomeric envelope proteins.
20

BACKGROUND OF THE INVENTION

 Acquired immune deficiency syndrome (AIDS) is recognized as one of the greatest health threats facing modern medicine. There is, as yet, no cure for this
25 disease.

In 1983-1984, three groups independently identified the suspected etiological agent of AIDS. See, e.g., Barre-Sinoussi et al. (1983) *Science* 220:868-871; Montagnier et al., in *Human T-Cell Leukemia Viruses* (Gallo, Essex & Gross, eds., 1984); Vilmer et al. (1984) *The Lancet* 1:753; Popovic et al. (1984) *Science* 224:497-500; Levy et al. (1984) *Science* 225:840-842. These isolates were variously called lymphadenopathy-associated virus (LAV), human T-cell lymphotropic virus type III (HTLV-III), or AIDS-associated retrovirus (ARV). All of these isolates are strains of the same virus, and were later collectively named Human Immunodeficiency Virus (HIV). With the isolation of a related AIDS-causing virus, the strains originally called HIV are now termed HIV-1 and the related virus is called HIV-2 See, e.g., Guyader et al. (1987) *Nature* 326:662-669; Brun-Vezinet et al. (1986) *Science* 233:343-346; Clavel et al. (1986) *Nature* 324:691-695.

A great deal of information has been gathered about the HIV virus, however, to date an effective vaccine has not been identified. Several targets for vaccine development have been examined including the *env*, *Gag*, *pol* and *tat* gene products encoded by HIV.

Haas, et al., (*Current Biology* 6(3):315-324, 1996) suggested that selective codon usage by HIV-1 appeared to account for a substantial fraction of the inefficiency of viral protein synthesis. Andre, et al., (*J. Virol.* 72(2):1497-1503, 1998) described an increased immune response elicited by DNA vaccination employing a synthetic gp120 sequence with optimized codon usage. Schneider, et al., (*J Virol.* 71(7):4892-4903, 1997) discuss inactivation

of inhibitory (or instability) elements (INS) located within the coding sequences of the Gag and Gag-protease coding sequences.

The *Gag* proteins of HIV-1 are necessary for the assembly of virus-like particles. HIV-1 *Gag* proteins are involved in many stages of the life cycle of the virus including, assembly, virion maturation after particle release, and early post-entry steps in virus replication. The roles of HIV-1 *Gag* proteins are numerous and complex (Freed, E.O., *Virology* 251:1-15, 1998).

Wolf, et al., (PCT International Application, WO 96/30523, published 3 October 1996; European Patent Application, Publication No. 0 449 116 A1, published 2 October 1991) have described the use of altered pr55 *Gag* of HIV-1 to act as a non-infectious retroviral-like particulate carrier, in particular, for the presentation of immunologically important epitopes. Wang, et al., (*Virology* 200:524-534, 1994) describe a system to study assembly of HIV *Gag*- β -galactosidase fusion proteins into virions. They describe the construction of sequences encoding HIV *Gag*- β -galactosidase fusion proteins, the expression of such sequences in the presence of HIV *Gag* proteins, and assembly of these proteins into virus particles.

Recently, Shiver, et al., (PCT International Application, WO 98/34640, published 13 August 1998) described altering HIV-1 (CAM1) *Gag* coding sequences to produce synthetic DNA molecules encoding HIV *Gag* and modifications of HIV *Gag*. The codons of the synthetic molecules were codons preferred by a projected host cell.

The envelope protein of HIV-1 is a glycoprotein of about 160 kD (gp160). During virus infection of the host

cell, gp160 is cleaved by host cell proteases to form gp120 and the integral membrane protein, gp41. The gp41 portion is anchored in (and spans) the membrane bilayer of virion, while the gp120 segment protrudes into the surrounding environment. As there is no covalent attachment between gp120 and gp41, free gp120 is released from the surface of virions and infected cells.

Haas, et al., (*Current Biology* 6(3):315-324, 1996) suggested that selective codon usage by HIV-1 appeared to account for a substantial fraction of the inefficiency of viral protein synthesis. Andre, et al., (*J. Virol.* 72(2):1497-1503, 1998) described an increased immune response elicited by DNA vaccination employing a synthetic gp120 sequence with optimized codon usage.

SUMMARY OF THE INVENTION

The present invention relates to improved expression of HIV *Env*-, *tat*-, *pol*-, *prot*-, reverse transcriptase, or *Gag*-containing polypeptides and production of virus-like particles.

In one embodiment the present invention includes an expression cassette, comprising a polynucleotide encoding an HIV *Gag* polypeptide comprising a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:20. In certain embodiments, the polynucleotide sequence encoding said *Gag* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:9 or SEQ ID NO:4. The expression cassettes may further include a polynucleotide sequence encoding an HIV protease polypeptide, for example a nucleotide sequence having at least 90% sequence identity to a sequence selected

from the group consisting of: SEQ ID NO:5, SEQ ID NO:78,
and SEQ ID NO:79. The expression cassettes may further
include a polynucleotide sequence encoding an HIV reverse
transcriptase polypeptide, for example a sequence having at
least 90% sequence identity to a sequence selected from the
group consisting of: SEQ ID NO:80, SEQ ID NO:81, SEQ ID
NO:82, SEQ ID NO:83, and SEQ ID NO:84. The expression
cassettes may further include a polynucleotide sequence
encoding an HIV *tat* polypeptide, for example a sequence
selected from the group consisting of: SEQ ID NO:87, SEQ ID
NO:88, and SEQ ID NO:89. The expression cassettes may
further include a polynucleotide sequence encoding an HIV
polymerase polypeptide, for example a sequence having at
least 90% sequence identity to the sequence presented as SEQ
ID NO:6. The expression cassettes may include a
polynucleotide sequence encoding an HIV *polymerase*
polypeptide, wherein (i) the nucleotide sequence encoding
said polypeptide comprises a sequence having at least 90%
sequence identity to the sequence presented as SEQ ID NO:4,
and (ii) wherein the sequence is modified by deletions of
coding regions corresponding to reverse transcriptase and
integrase. The expression cassettes described above may
preserves T-helper cell and CTL epitopes. The expression
cassettes may further include a polynucleotide sequence
encoding an HCV core polypeptide, for example a sequence
having at least 90% sequence identity to the sequence
presented as SEQ ID NO:7.

In another aspect, the invention includes an expression
cassette, comprising a polynucleotide sequence encoding a
polypeptide including an HIV *Env* polypeptide, wherein the
polynucleotide sequence encoding said *Env* polypeptide

comprises a sequence having at least 90% sequence identity to SEQ ID NO:71 (Figure 58) or SEQ ID NO:72 (Figure 59). In certain embodiments, the Env expression cassettes includes sequences flanking a V1 region but have a deletion in the V1 region itself, for example the sequence presented as SEQ ID NO:65 (Figure 52, gp160.modUS4.delV1). In certain embodiments, the Env expression cassettes, include sequences flanking a V2 region but have a deletion in the V2 region itself, for example the sequences shown in SEQ ID NO:60 (Figure 47); SEQ ID NO:66 (Figure 53); SEQ ID NO:34 (Figure 20); SEQ ID NO:37 (Figure 24); SEQ ID NO:40 (Figure 27); SEQ ID NO:43 (Figure 30); SEQ ID NO:46 (Figure 33); SEQ ID NO:76 (Figure 64) and SEQ ID NO:49 (Figure 36). In certain embodiments, the Env expression cassettes include sequences flanking a V1/V2 region but have a deletion in the V1/V2 region itself, for example, SEQ ID NO:59 (Figure 46); SEQ ID NO:61 (Figure 48); SEQ ID NO:67 (Figure 54); SEQ ID NO:75 (Figure 63); SEQ ID NO:35 (Figure 21); SEQ ID NO:38 (Figure 25); SEQ ID NO:41 (Figure 28); SEQ ID NO:44 (Figure 31); SEQ ID NO:47 (Figure 34) and SEQ ID NO:50 (Figure 37). The Env-encoding expression cassettes may also include a mutated cleavage site that prevents the cleavage of a gp140 polypeptide into a gp120 polypeptide and a gp41 polypeptide, for example, SEQ ID NO:57 (Figure 44); SEQ ID NO:61 (Figure 48); SEQ ID NO:63 (Figure 50); SEQ ID NO:39 (Figure 26); SEQ ID NO:40 (Figure 27); SEQ ID NO:41 (Figure 28); SEQ ID NO:42 (Figure 29); SEQ ID NO:43 (Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45 (Figure 32); SEQ ID NO:46 (Figure 33); and SEQ ID NO:47 (Figure 34). The Env expression cassettes may include a gp160 Env polypeptide or a polypeptide derived

from a gp160 *Env* polypeptide, for example SEQ ID NO:64
(Figure 51); SEQ ID NO:65 (Figure 52); SEQ ID NO:66 (Figure
53); SEQ ID NO:67 (Figure 54); SEQ ID NO:68 (Figure 55); SEQ
ID NO:75 (Figure 63); SEQ ID NO:73 (Figure 61); SEQ ID NO:48
5 (Figure 35); SEQ ID NO:49 (Figure 36); SEQ ID NO:50 (Figure
37); SEQ ID NO:76 (Figure 64); and SEQ ID NO:74 (Figure 62).

The *Env* expression cassettes may include a gp140 *Env*
polypeptide or a polypeptide derived from a gp140 *Env*
polypeptide, for example SEQ ID NO:56 (Figure 43); SEQ ID
10 NO:57 (Figure 44); SEQ ID NO:58 (Figure 45); SEQ ID NO:59
(Figure 46); SEQ ID NO:60 (Figure 47); SEQ ID NO:61 (Figure
48); SEQ ID NO:62 (Figure 49); SEQ ID NO:63 (Figure 50); SEQ
ID NO:36 (Figure 23); SEQ ID NO:37 (Figure 24); SEQ ID NO:38
(Figure 25); SEQ ID NO:39 (Figure 26); SEQ ID NO:40 (Figure
15 27); SEQ ID NO:41 (Figure 28); SEQ ID NO:42 (Figure 29); SEQ
ID NO:43 (Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45
(Figure 32); SEQ ID NO:46 (Figure 33); and SEQ ID NO:47
(Figure 34). The *Env* expression cassettes may also include
a gp120 *Env* polypeptide or a polypeptide derived from a
20 gp120 *Env* polypeptide, for example SEQ ID NO:54 (Figure 41);
and SEQ ID NO:55 (Figure 42); SEQ ID NO:33 (Figure 19); SEQ
ID NO:34 (Figure 20); and SEQ ID NO:35 (Figure 21). The *Env*
expression cassettes may include an *Env* polypeptide lacking
the amino acids corresponding to residues 128 to about 194,
25 relative to strains SF162 or US4, for example, SEQ ID NO:55
(Figure 42); SEQ ID NO:62 (Figure 49); SEQ ID NO:63 (Figure
50); and SEQ ID NO:68 (Figure 55).

In another aspect, the invention includes a recombinant
expression system for use in a selected host cell,
30 comprising, one or more of the expression cassettes

described herein operably linked to control elements compatible with expression in the selected host cell. The expression cassettes may be included on one or on multiple vectors and may use the same or different promoters.

5 Exemplary control elements include a transcription promoter (e.g., CMV, CMV+intron A, SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein), a transcription enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and
10 translation termination sequences.

In another aspect, the invention includes a recombinant expression system for use in a selected host cell, comprising, any one of the expression cassettes described herein operably linked to control elements compatible with
15 expression in the selected host cell. Exemplary control elements include, but are not limited to, a transcription promoter (e.g., CMV, CMV+intron A, SV40, RSV, HIV-LTR, MMLV-LTR, and metallothionein), a transcription enhancer element, a transcription termination signal, polyadenylation
20 sequences, sequences for optimization of initiation of translation, and translation termination sequences.

In yet another aspect, the invention includes a cell comprising one or more of the expression cassettes described herein operably linked to control elements compatible with
25 expression in the cell. The cell can be, for example, a mammalian cell (e.g., BHK, VERO, HT1080, 293, RD, COS-7, or CHO cells), an insect cell (e.g., *Trichoplusia ni* (Tn5) or Sf9), a bacterial cell, a plant cell, a yeast cell, an antigen presenting cell (e.g., primary, immortalized or
30 tumor-derived lymphoid cells such as macrophages, monocytes,

dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof).

In another aspect, the invention includes methods for producing a polypeptide including HIV *Gag*-, *prot*-, *pol*-,
5 *reverse transcriptase*, *Env*- or *Tat*-containing polypeptide sequences, said method comprising, incubating the cells comprising one or more the expression cassettes describe herein, under conditions for producing said polypeptide.

In yet another aspect, the invention includes
10 compositions for generating an immunological response, comprising one or more of the expression cassettes described herein. In certain embodiments, the compositions also include an adjuvant.

In a still further aspect, the invention includes
15 methods of generating an immune response in a subject, comprising introducing a composition comprising one or more of the expression cassettes described herein into the subject under conditions that are compatible with expression of said expression cassette in the subject. In certain
20 embodiments, the expression cassette is introduced using a gene delivery vector. More than one expression cassette may be introduced using one or more gene delivery vectors.

In yet another aspect, the invention includes a purified polynucleotide comprising a polynucleotide sequence
25 encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:71 (Figure 58) or SEQ ID NO:72 (Figure 59). Further exemplary purified
30 polynucleotide sequences were presented above.

The polynucleotides of the present invention can be produced by recombinant techniques, synthetic techniques, or combinations thereof.

In another embodiment, the invention includes a method
5 for producing a polypeptide including HIV *Gag* polypeptide sequences, where the method comprises incubating any of the above cells containing an expression cassette of interest under conditions for producing the polypeptide.

The invention further includes, a method for producing
10 virus-like particles (VLPs) where the method comprises incubating any of the above-described cells containing an expression cassette of interest under conditions for producing VLPs.

In another aspect the invention includes a method for
15 producing a composition of virus-like particles (VLPs) where, any of the above-described cells containing an expression cassette of interest are incubated under conditions for producing VLPs, and the VLPs are substantially purified to produce a composition of VLPs.

20 In a further embodiment of the present invention, packaging cell lines are produced using the expression cassettes of the present invention. For example, a cell line useful for packaging lentivirus vectors comprises suitable host cells that have an expression vector
25 containing an expression cassette of the present invention wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell. In a preferred embodiment, such host cells may be transfected with one or more expression cassettes having a
30 polynucleotide sequence that encodes an HIV *polymerase*

polypeptide or polypeptides derived therefrom, for example, where the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:6. Further, the HIV
5 *polymerase* polypeptide may be modified by deletions of coding regions corresponding to reverse transcriptase and integrase. Such a polynucleotide sequence may preserve T-helper cell and CTL epitopes, for example when used in a vaccine application. In addition, the polynucleotide
10 sequence may also include other polypeptides. Further, polynucleotide sequences encoding additional polypeptides whose expression are useful for packaging cell line function may also be utilized.

In another aspect, the present invention includes a
15 gene delivery or vaccine vector for use in a subject, where the vector is a suitable gene delivery vector for use in the subject, and the vector comprises one or more of any of the expression cassettes of the present invention where the polynucleotide sequences of interest are operably linked to
20 control elements compatible with expression in the subject. Such gene delivery vectors can be used in a method of DNA immunization of a subject, for example, by introducing a gene delivery vector into the subject under conditions that are compatible with expression of the expression cassette in
25 the subject. Gene delivery vectors useful in the practice of the present invention include, but are not limited to, nonviral vectors, bacterial plasmid vectors, viral vectors, particulate carriers (where the vector is coated on a polylactide co-glycolide particles, gold or tungsten
30 particle, for example, the coated particle can be delivered

to a subject cell using a gene gun), liposome preparations, and viral vectors (e.g., vectors derived from alphaviruses, pox viruses, and vaccinia viruses, as well as, retroviral vectors, including, but not limited to, lentiviral vectors).

- 5 Alphavirus-derived vectors include, for example, an alphavirus cDNA construct, a recombinant alphavirus particle preparation and a eukaryotic layered vector initiation system. In one embodiment, the subject is a vertebrate, preferably a mammal, and in a further embodiment the subject
10 is a human.

The invention further includes a method of generating an immune response in a subject, where cells of a subject are transfected with any of the above-described gene delivery vectors (e.g., alphavirus constructs; alphavirus
15 cDNA constructs; eukaryotic layered vector initiation systems (see, e.g., U.S. Patent Number 5,814,482 for description of suitable eukaryotic layered vector initiation systems); alphavirus particle preparations; etc.) under conditions that permit the expression of a selected
20 polynucleotide and production of a polypeptide of interest (i.e., encoded by any expression cassette of the present invention), thereby eliciting an immunological response to the polypeptide. Transfection of the cells may be performed ex vivo and the transfected cells are reintroduced into the
25 subject. Alternately, or in addition, the cells may be transfected in vivo in the subject. The immune response may be humoral and/or cell-mediated (cellular).

Further embodiments of the present invention include purified polynucleotides. In one embodiment, the purified
30 polynucleotide comprises a polynucleotide sequence having at

least 90% sequence identity to the sequence presented as SEQ ID NO:20, and complements thereof. In another embodiment, the purified polynucleotide comprises a polynucleotide sequence encoding an HIV Gag polypeptide, wherein the polynucleotide sequence comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:20, and complements thereof. In still another embodiment, the purified polynucleotide comprises a polynucleotide sequence encoding an HIV Gag polypeptide, wherein the polynucleotide sequence comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:9, and complements thereof. In further embodiments the polynucleotide sequence comprises a sequence having at least 90% sequence identity to one of the following sequences: SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and complements thereof.

The polynucleotides of the present invention can be produced by recombinant techniques, synthetic techniques, or combinations thereof.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the locations of the inactivation sites for the native HIV-1SF2 Gag protein coding sequence.

Figure 2 shows the locations of the inactivation sites for the native HIV-1SF2 Gag-protease protein coding sequence.

Figures 3A and 3B show electron micrographs of virus-like particles. Figure 3A shows immature p55Gag virus-like particles in COS-7 cells transfected with a synthetic HIV-1_{SF2} gag construct while Figure 3B shows mature (arrows) and immature VLP in cells transfected with a modified HIV-1_{SF2} gagprotease construct (GP2, SEQ ID NO:70). Transfected cells were fixed at 24 h (gag) or 48 h (gagprotease) post-transfection and subsequently analyzed by electron microscopy (magnification at 100,000X). Cells transfected with vector alone (pCMVKm2) served as negative control (data not shown).

Figure 4 presents an image of samples from a series of fractions which were electrophoresed on an 8-16% SDS polyacrylamide gel and the resulting bands visualized by commassie blue staining. The results show that the native p55 Gag virus-like particles (VLPs) banded at a sucrose density of range of 1.15 - 1.19 g/ml with the peak at approximately 1.17 g/ml.

Figure 5 presents an image similar to Figure 4 where the analysis was performed using Gag VLPs produced by a synthetic Gag expression cassette.

Figure 6 presents a comparison of the total amount of purified HIV p55 Gag from several preparations obtained from two baculovirus expression cassettes encoding native and modified Gag.

Figure 7 presents an alignment of modified coding sequences of the present invention including a synthetic Gag expression cassette (SEQ ID NO:4), a synthetic Gag-protease expression cassette (SEQ ID NO:5), and a synthetic Gag-polymerase expression cassette (SEQ ID NO:6). A common

region (Gag-common; SEQ ID NO:9) extends from position 1 to position 1262.

Figure 8 presents an image of wild-type Gag-HCV core expression samples from a series of fractions which were electrophoresed on an 8-16% SDS polyacrylamide gel and the resulting bands visualized by commassie staining.

Figure 9 shows the results of Western blot analysis of the gel shown presented in Figure 8.

Figure 10 presents results similar to those shown in Figure 9. The results in Figure 10 indicate that the main HCV Core-specific reactivity migrates at an approximate molecular weight of 72,000 kD, which is in accordance with the predicted molecular weight of the Gag-HCV core chimeric protein.

Figures 11A to 11D present a comparison of AT content, in percent, of cDNAs corresponding to an unstable human mRNA (human IFN γ mRNA; 11A), wild-type HIV Gag native RNA (11B), a stable human mRNA (human GAPDH mRNA; 11C), and synthetic HIV Gag RNA (11D).

Figure 12 shows the location of the inactivation sites for the native HIV-1SF2 Gag-polymerase sequence.

Figure 13A presents a vector map of pESN2dhfr.

Figure 13B presents a map of the pCMVIII vector.

Figure 14 presents a vector map of pCMV-LINK.

Figure 15 presents a schematic diagram showing the relationships between the following forms of the HIV Env polypeptide: gp160, gp140, gp120, and gp41.

Figure 16 depicts the nucleotide sequence of wild-type gp120 from SF162 (SEQ ID NO:30).

Figure 17 depicts the nucleotide sequence of the wild-type gp140 from SF162 (SEQ ID NO:31).

Figure 18 depicts the nucleotide sequence of the wild-type gp160 from SF162 (SEQ ID NO:32).

Figure 19 depicts the nucleotide sequence of the construct designated gp120.modSF162 (SEQ ID NO:33).

5 Figure 20 depicts the nucleotide sequence of the construct designated gp120.modSF162.delV2 (SEQ ID NO:34).

Figure 21 depicts the nucleotide sequence of the construct designated gp120.modSF162.delV1/V2 (SEQ ID NO:35).

10 Figures 22A-H show the percent A-T content over the length of the sequences for IFN γ (Figures 2C and 2G); native gp160 Env US4 and SF162 (Figures 2A and 2E, respectively); GAPDH (Figures 2D and 2H); and the synthetic gp160 Env for US4 and SF162 (Figures 2B and 2F, respectively).

15 Figure 23 depicts the nucleotide sequence of the construct designated gp140.modSF162 (SEQ ID NO:36).

Figure 24 depicts the nucleotide sequence of the construct designated gp140.modSF162.delV2 (SEQ ID NO:37).

Figure 25 depicts the nucleotide sequence of the construct designated gp140.modSF162.delV1/V2 (SEQ ID NO:38).

20 Figure 26 depicts the nucleotide sequence of the construct designated gp140.mut.modSF162 (SEQ ID NO:39).

Figure 27 depicts the nucleotide sequence of the construct designated gp140.mut.modSF162.delV2 (SEQ ID NO:40).

25 Figure 28 depicts the nucleotide sequence of the construct designated gp140.mut.modSF162.delV1/V2 (SEQ ID NO:41).

Figure 29 depicts the nucleotide sequence of the construct designated gp140.mut7.modSF162 (SEQ ID NO:42).

30 Figure 30 depicts the nucleotide sequence of the construct designated gp140.mut7.modSF162.delV2 (SEQ ID NO:43).

Figure 31 depicts the nucleotide sequence of the construct designated gp140.mut7.modSF162.delV1/V2 (SEQ ID NO:44).

5 Figure 32 depicts the nucleotide sequence of the construct designated gp140.mut8.modSF162 (SEQ ID NO:45).

Figure 33 depicts the nucleotide sequence of the construct designated gp140.mut8.modSF162.delV2 (SEQ ID NO:46).

10 Figure 34 depicts the nucleotide sequence of the construct designated gp140.mut8.modSF162.delV1/V2 (SEQ ID NO:47).

Figure 35 depicts the nucleotide sequence of the construct designated gp160.modSF162 (SEQ ID NO:48).

15 Figure 36 depicts the nucleotide sequence of the construct designated gp160.modSF162.delV2 (SEQ ID NO:49).

Figure 37 depicts the nucleotide sequence of the construct designated gp160.modSF162.delV1/V2 (SEQ ID NO:50).

Figure 38 depicts the nucleotide sequence of the wild-type gp120 from US4 (SEQ ID NO:51).

20 Figure 39 depicts the nucleotide sequence of the wild-type gp140 from US4 (SEQ ID NO:52).

Figure 40 depicts the nucleotide sequence of the wild-type gp160 from US4 (SEQ ID NO:53).

25 Figure 41 depicts the nucleotide sequence of the construct designated gp120.modUS4 (SEQ ID NO:54).

Figure 42 depicts the nucleotide sequence of the construct designated gp120.modUS4.del 128-194 (SEQ ID NO:55).

30 Figure 43 depicts the nucleotide sequence of the construct designated gp140.modUS4 (SEQ ID NO:56).

Figure 45 depicts the nucleotide sequence of the construct designated gp140.TM.modUS4 (SEQ ID NO:58).

Figure 47 depicts the nucleotide sequence of the construct designated gp140.modUS4.delV2 (SEQ ID NO:60).

Figure 49 depicts the nucleotide sequence of the construct designated gp140.modUS4.del 128-194 (SEQ ID NO:62).

Figure 51 depicts the nucleotide sequence of the construct designated gp160.modUS4 (SEQ ID NO:64).

Figure 53 depicts the nucleotide sequence of the construct designated gp160.modUS4.delV2 (SEQ ID NO:66).

Figure 55 depicts the nucleotide sequence of the construct designated gp160.modUS4.del 128-194 (SEQ ID NO:68).

Figure 57 depicts the nucleotide sequence of the common region of Env from wild-type SF162 (SEQ ID NO:70).

Figure 58 depicts the nucleotide sequence of synthetic sequences corresponding to the common region of Env from US4
5 (SEQ ID NO:71).

Figure 59 depicts the nucleotide sequence of synthetic sequences corresponding to the common region of Env from SF162 (SEQ ID NO:72).

Figure 60 presents a schematic representation of an Env
10 polypeptide purification strategy.

Figure 61 depicts the nucleotide sequence of the bicistronic construct designated gp160.modUS4.Gag.modSF2 (SEQ ID NO:73).

Figure 62 depicts the nucleotide sequence of the
15 bicistronic construct designated gp160.modSF162.Gag.modSF2 (SEQ ID NO:74).

Figure 63 depicts the nucleotide sequence of the bicistronic construct designated gp160.modUS4.-delV1/V2.Gag.modSF2 (SEQ ID NO:75).

Figure 64 depicts the nucleotide sequence of the
20 bicistronic construct designated gp160.modSF162.delV2.Gag.modSF2 (SEQ ID NO:76).

Figures 65A-65F show micrographs of 293T cells transfected with the following polypeptide encoding
25 sequences: Figure 65A, gag.modSF2; Figure 65B, gp160.modUS4; Figure 65C, gp160.modUS4.delV1/V2.gag.modSF2 (bicistronic Env and Gag); Figures 65D and 65E, gp160.modUS4.delV1/V2 and gag.modSF2; and Figure 65F, gp120.modSF162.delV2 and gag.modSF2.

Figures 66A and 66B present alignments of selected
30 modified coding sequences of the present invention including

a common region defined for each group of synthetic *Env* expression cassettes. Figure 66A presents alignments of modified SF162 sequences. Figure 66B presents alignments of modified US4 sequences. The SEQ ID NOs for these sequences are presented in Tables 1A and 1B.

Figure 67 shows the ELISA titers (binding antibodies) obtained in two rhesus macaques (H445, lines with solid black dots; and J408, lines with open squares). The y-axis is the end-point gp140 ELISA titers and the x-axis shows weeks post-immunization. The dashed lines at 0, 4, and 8 weeks represent DNA immunizations. The alternating dash/dotted line at 27 weeks indicates a DNA plus protein boost immunization.

Figure 68 (SEQ ID NO:77) depicts the wild-type nucleotide sequence of Gag reverse transcriptase from SF2.

Figure 69 (SEQ ID NO:78) depicts the nucleotide sequence of the construct designated GP1.

Figure 70 (SEQ ID NO:79) depicts the nucleotide sequence of the construct designated GP2.

Figure 71 (SEQ ID NO:80) depicts the nucleotide sequence of the construct designated FS(+).protinact.RTopt.YM. FS(+) indicates that there is a frameshift in the GagPol coding sequence.

Figure 72 (SEQ ID NO:81) depicts the nucleotide sequence of the construct designated FS(+).protinact.RTopt.YMWM.

Figure 73 (SEQ ID NO:82) depicts the nucleotide sequence of the construct designated FS(-).protmod.RTopt.YM. FS(-) indicates that there is no frameshift in the GagPol coding sequence.

Figure 74 (SEQ ID NO:83) depicts the nucleotide sequence of the construct designated FS(-).protmod.RTopt.YMWM.

5 Figure 75 (SEQ ID NO:84) depicts the nucleotide sequence of the construct designated FS(-).protmod.RTopt(+).

Figure 76 (SEQ ID NO:85) depicts the nucleotide sequence of wild type Tat from isolate SF162.

Figure 77 (SEQ ID NO:86) depicts the amino acid sequence of the tat polypeptide.

10 Figure 78 (SEQ ID NO:87) depicts the nucleotide sequence of a synthetic Tat construct designated Tat.SF162.opt.

15 Figure 79 (SEQ ID NO:88) depicts the nucleotide sequence of a synthetic Tat construct designated tat.cys22.sf162.opt. The construct encodes a tat polypeptide in which the cystein residue at position 22 of the wild type Tat polypeptide is replaced by a glycine residue.

20 Figures 80A to 80E are an alignment of the nucleotide sequences of the constructs designated Gag.mod.SF2, GP1 (SEQ ID NO:78), and GP2 (SEQ ID NO:79).

25 Figure 81 (SEQ ID NO:89) depicts the nucleotide sequence of the construct designated tataminoSF162.opt, which encodes the amino terminus of that tat protein. The codon encoding the cystein-22 residue is underlined.

Figure 82 (SEQ ID NO:90) depicts the amino acid sequence of the polypeptide encoded by the construct designated tat.cys22.SF162.opt (SEQ ID NO:88).

DETAILED DESCRIPTION OF THE INVENTION

5 The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, 10 Inc.); and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds., 1986, Blackwell Scientific Publications); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Short Protocols in Molecular Biology*, 4th ed. (Ausubel et al. 15 eds., 1999, John Wiley & Sons); *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream et al., eds., 1998, Academic Press); *PCR (Introduction to Biotechniques Series)*, 2nd ed. (Newton & Graham eds., 1997, Springer Verlag).

20 All publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural 25 references unless the content clearly dictates otherwise. Thus, for example, reference to "an antigen" includes a mixture of two or more such agents.

1. DEFINITIONS

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

5 "Synthetic" sequences, as used herein, refers to Env-, tat- or Gag-encoding polynucleotides whose expression has been optimized as described herein, for example, by codon substitution, deletions, replacements and/or inactivation of inhibitory sequences. "Wild-type" or "native" sequences, as
10 used herein, refers to polypeptide encoding sequences that are essentially as they are found in nature, e.g., Gag encoding sequences as found in the isolate HIV-1SF2 or Env encoding sequences as found in the isolates HIV-1SF162 or HIV1US4.

15 As used herein, the term "virus-like particle" or "VLP" refers to a nonreplicating, viral shell, derived from any of several viruses discussed further below. VLPs are generally composed of one or more viral proteins, such as, but not limited to those proteins referred to as capsid,
20 coat, shell, surface and/or envelope proteins, or particle-forming polypeptides derived from these proteins. VLPs can form spontaneously upon recombinant expression of the protein in an appropriate expression system. Methods for producing particular VLPs are known in the art and discussed
25 more fully below. The presence of VLPs following recombinant expression of viral proteins can be detected using conventional techniques known in the art, such as by electron microscopy, biophysical characterization, and the like. See, e.g., Baker et al., *Biophys. J.* (1991) 60:1445-
30 1456; Hagensee et al., *J. Virol.* (1994) 68:4503-4505. For

example, VLPs can be isolated by density gradient centrifugation and/or identified by characteristic density banding (e.g., Example 7). Alternatively, cryoelectron microscopy can be performed on vitrified aqueous samples of the VLP preparation in question, and images recorded under appropriate exposure conditions.

By "particle-forming polypeptide" derived from a particular viral protein is meant a full-length or near full-length viral protein, as well as a fragment thereof, or a viral protein with internal deletions, which has the ability to form VLPs under conditions that favor VLP formation. Accordingly, the polypeptide may comprise the full-length sequence, fragments, truncated and partial sequences, as well as analogs and precursor forms of the reference molecule. The term therefore intends deletions, additions and substitutions to the sequence, so long as the polypeptide retains the ability to form a VLP. Thus, the term includes natural variations of the specified polypeptide since variations in coat proteins often occur between viral isolates. The term also includes deletions, additions and substitutions that do not naturally occur in the reference protein, so long as the protein retains the ability to form a VLP. Preferred substitutions are those which are conservative in nature, i.e., those substitutions that take place within a family of amino acids that are related in their side chains. Specifically, amino acids are generally divided into four families: (1) acidic -- aspartate and glutamate; (2) basic -- lysine, arginine, histidine; (3) non-polar -- alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar -- glycine, asparagine, glutamine, cystine, serine threonine, tyrosine. Phenylalanine,

tryptophan, and tyrosine are sometimes classified as aromatic amino acids.

5 An "antigen" refers to a molecule containing one or more epitopes (either linear, conformational or both) that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term is used interchangeably with the term "immunogen." Normally, a B-cell epitope will include at least about 5 amino acids but can be as small as 3-4 amino acids. A T-cell epitope, such as a CTL epitope, will include at least about 7-9 amino acids, and a helper T-cell epitope at least about 12-20 amino acids. Normally, an epitope will include between about 7 and 15 amino acids, such as, 9, 10, 12 or 15 amino acids. The term "antigen" denotes both subunit antigens, (i.e., antigens which are separate and discrete from a whole organism with which the antigen is associated in nature), as well as, killed, attenuated or inactivated bacteria, viruses, fungi, parasites or other microbes. Antibodies such as anti-idiotypic antibodies, or fragments thereof, and synthetic peptide mimotopes, which can mimic an antigen or antigenic determinant, are also captured under the definition of antigen as used herein. Similarly, an oligonucleotide or polynucleotide which expresses an antigen or antigenic determinant *in vivo*, such as in gene therapy and DNA immunization applications, is also included in the definition of antigen herein.

For purposes of the present invention, antigens can be derived from any of several known viruses, bacteria, parasites and fungi, as described more fully below. The term also intends any of the various tumor antigens. Furthermore, for purposes of the present invention, an

"antigen" refers to a protein which includes modifications, such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, so long as the protein maintains the ability to elicit an immunological response, as defined herein. These modifications may be deliberate, as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the antigens.

An "immunological response" to an antigen or composition is the development in a subject of a humoral and/or a cellular immune response to an antigen present in the composition of interest. For purposes of the present invention, a "humoral immune response" refers to an immune response mediated by antibody molecules, while a "cellular immune response" is one mediated by T-lymphocytes and/or other white blood cells. One important aspect of cellular immunity involves an antigen-specific response by cytolytic T-cells ("CTL"s). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the destruction of intracellular microbes, or the lysis of cells infected with such microbes. Another aspect of cellular immunity involves an antigen-specific response by helper T-cells. Helper T-cells act to help stimulate the function, and focus the activity of, nonspecific effector cells against cells displaying peptide antigens in association with MHC molecules on their surface. A "cellular immune response" also refers to the production of cytokines, chemokines and other such molecules produced by activated T-cells and/or other white blood cells, including those derived from CD4+ and CD8+ T-cells.

A composition or vaccine that elicits a cellular immune response may serve to sensitize a vertebrate subject by the presentation of antigen in association with MHC molecules at the cell surface. The cell-mediated immune response is
5 directed at, or near, cells presenting antigen at their surface. In addition, antigen-specific T-lymphocytes can be generated to allow for the future protection of an immunized host.

The ability of a particular antigen to stimulate a
10 cell-mediated immunological response may be determined by a number of assays, such as by lymphoproliferation (lymphocyte activation) assays, CTL cytotoxic cell assays, or by assaying for T-lymphocytes specific for the antigen in a sensitized subject. Such assays are well known in the art.
15 See, e.g., Erickson et al., *J. Immunol.* (1993) 151:4189-4199; Doe et al., *Eur. J. Immunol.* (1994) 24:2369-2376. Recent methods of measuring cell-mediated immune response include measurement of intracellular cytokines or cytokine secretion by T-cell populations, or by measurement of
20 epitope specific T-cells (e.g., by the tetramer technique) (reviewed by McMichael, A.J., and O'Callaghan, C.A., *J. Exp. Med.* 187(9)1367-1371, 1998; Mcheyzer-Williams, M.G., et al, *Immunol. Rev.* 150:5-21, 1996; Lalvani, A., et al, *J. Exp. Med.* 186:859-865, 1997).

25 Thus, an immunological response as used herein may be one which stimulates the production of CTLs, and/or the production or activation of helper T- cells. The antigen of interest may also elicit an antibody-mediated immune response. Hence, an immunological response may include one
30 or more of the following effects: the production of antibodies by B-cells; and/or the activation of suppressor

5 T-cells and/or $\gamma\delta$ T-cells directed specifically to an antigen or antigens present in the composition or vaccine of interest. These responses may serve to neutralize infectivity, and/or mediate antibody-complement, or antibody dependent cell cytotoxicity (ADCC) to provide protection to an immunized host. Such responses can be determined using standard immunoassays and neutralization assays, well known in the art.

10 An "immunogenic composition" is a composition that comprises an antigenic molecule where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigenic molecule of interest.

15 By "subunit vaccine" is meant a vaccine composition which includes one or more selected antigens but not all antigens, derived from or homologous to, an antigen from a pathogen of interest such as from a virus, bacterium, parasite or fungus. Such a composition is substantially free of intact pathogen cells or pathogenic particles, or the lysate of such cells or particles. Thus, a "subunit vaccine" can be prepared from at least partially purified (preferably substantially purified) immunogenic polypeptides from the pathogen, or analogs thereof. The method of obtaining an antigen included in the subunit vaccine can thus include standard purification techniques, recombinant production, or synthetic production.

25 "Substantially purified" general refers to isolation of a substance (compound, polynucleotide, protein, polypeptide, polypeptide composition) such that the substance comprises the majority percent of the sample in which it resides. Typically in a sample a substantially purified component comprises 50%, preferably 80%-85%, more preferably 90-95% of

the sample. Techniques for purifying polynucleotides and polypeptides of interest are well-known in the art and include, for example, ion-exchange chromatography, affinity chromatography and sedimentation according to density.

5 A "coding sequence" or a sequence which "encodes" a selected polypeptide, is a nucleic acid molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences (or "control
10 elements"). The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from viral, procaryotic or eucaryotic mRNA, genomic DNA
15 sequences from viral or procaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence may be located 3' to the coding sequence.

Typical "control elements", include, but are not limited to, transcription promoters, transcription enhancer
20 elements, transcription termination signals, polyadenylation sequences (located 3' to the translation stop codon), sequences for optimization of initiation of translation (located 5' to the coding sequence), and translation termination sequences, see e.g., McCaughan et al. (1995)
25 *PNAS USA* 92:5431-5435; Kochetov et al (1998) *FEBS Letts.* 440:351-355.

A "nucleic acid" molecule can include, but is not limited to, procaryotic sequences, eucaryotic mRNA, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic
30 (e.g., mammalian) DNA, and even synthetic DNA sequences.

The term also captures sequences that include any of the known base analogs of DNA and RNA.

5 "Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, a given promoter operably linked to a coding sequence is capable of effecting the expression of the coding sequence when the proper enzymes are present. The promoter need not be contiguous with the coding sequence, so long as it functions to direct
10 the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between the promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

15 "Recombinant" as used herein to describe a nucleic acid molecule means a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of the polynucleotide with which it is associated in
20 nature; and/or (2) is linked to a polynucleotide other than that to which it is linked in nature. The term "recombinant" as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide. "Recombinant host cells," "host cells,"
25 "cells," "cell lines," "cell cultures," and other such terms denoting procaryotic microorganisms or eucaryotic cell lines cultured as unicellular entities, are used interchangeably, and refer to cells which can be, or have been, used as recipients for recombinant vectors or other transfer DNA,
30 and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in

5 morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation. Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide sequence encoding a desired peptide, are included in the progeny intended by this definition, and are covered by the above terms.

10 Techniques for determining amino acid sequence "similarity" are well known in the art. In general, "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively.

25 Two or more polynucleotide sequences can be compared by determining their "percent identity." Two or more amino acid sequences likewise can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or peptide sequences, is generally described as the number of exact matches between two aligned

30

sequences divided by the length of the shorter sequence and multiplied by 100. An approximate alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981). This algorithm can be extended to use with peptide sequences using the scoring matrix developed by Dayhoff, *Atlas of Protein Sequences and Structure*, M.O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, *Nucl. Acids Res.* 14(6):6745-6763 (1986). An implementation of this algorithm for nucleic acid and peptide sequences is provided by the Genetics Computer Group (Madison, WI) in their BestFit utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, WI). Other equally suitable programs for calculating the percent identity or similarity between sequences are generally known in the art.

For example, percent identity of a particular nucleotide sequence to a reference sequence can be determined using the homology algorithm of Smith and Waterman with a default scoring table and a gap penalty of six nucleotide positions. Another method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages, the Smith-Waterman algorithm can be employed where default

parameters are used for the scoring table (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated, the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, such as the alignment program BLAST, which can also be used with default parameters. For example, BLASTN and BLASTP can be used with the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address: <http://www.ncbi.nlm.gov/cgi-bin/BLAST>.

One of skill in the art can readily determine the proper search parameters to use for a given sequence in the above programs. For example, the search parameters may vary based on the size of the sequence in question. Thus, for example, a representative embodiment of the present invention would include an isolated polynucleotide having X contiguous nucleotides, wherein (i) the X contiguous nucleotides have at least about 50% identity to Y contiguous nucleotides derived from any of the sequences described herein, (ii) X equals Y, and (iii) X is greater than or equal to 6 nucleotides and up to 5000 nucleotides, preferably greater than or equal to 8 nucleotides and up to 5000 nucleotides, more preferably 10-12 nucleotides and up to 5000 nucleotides, and even more preferably 15-20

nucleotides, up to the number of nucleotides present in the full-length sequences described herein (e.g., see the Sequence Listing and claims), including all integer values falling within the above-described ranges.

5 The synthetic expression cassettes (and purified polynucleotides) of the present invention include related polynucleotide sequences having about 80% to 100%, greater than 80-85%, preferably greater than 90-92%, more preferably greater than 95%, and most preferably greater than 98%
10 sequence (including all integer values falling within these described ranges) identity to the synthetic expression cassette sequences disclosed herein (for example, to the sequences presented in Tables 1A and 1B) when the sequences of the present invention are used as the query sequence.

15 Two nucleic acid fragments are considered to "selectively hybridize" as described herein. The degree of sequence identity between two nucleic acid molecules affects the efficiency and strength of hybridization events between such molecules. A partially identical nucleic acid sequence
20 will at least partially inhibit a completely identical sequence from hybridizing to a target molecule. Inhibition of hybridization of the completely identical sequence can be assessed using hybridization assays that are well known in the art (e.g., Southern blot, Northern blot, solution
25 hybridization, or the like, see Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, (1989) Cold Spring Harbor, N.Y.). Such assays can be conducted using varying degrees of selectivity, for example, using conditions varying from low to high stringency. If
30 conditions of low stringency are employed, the absence of

non-specific binding can be assessed using a secondary probe that lacks even a partial degree of sequence identity (for example, a probe having less than about 30% sequence identity with the target molecule), such that, in the
5 absence of non-specific binding events, the secondary probe will not hybridize to the target.

When utilizing a hybridization-based detection system, a nucleic acid probe is chosen that is complementary to a target nucleic acid sequence, and then by selection of
10 appropriate conditions the probe and the target sequence "selectively hybridize," or bind, to each other to form a hybrid molecule. A nucleic acid molecule that is capable of hybridizing selectively to a target sequence under "moderately stringent" typically hybridizes under conditions
15 that allow detection of a target nucleic acid sequence of at least about 10-14 nucleotides in length having at least approximately 70% sequence identity with the sequence of the selected nucleic acid probe. Stringent hybridization conditions typically allow detection of target nucleic acid
20 sequences of at least about 10-14 nucleotides in length having a sequence identity of greater than about 90-95% with the sequence of the selected nucleic acid probe. Hybridization conditions useful for probe/target hybridization where the probe and target have a specific
25 degree of sequence identity, can be determined as is known in the art (see, for example, Nucleic Acid Hybridization: A Practical Approach, editors B.D. Hames and S.J. Higgins, (1985) Oxford; Washington, DC; IRL Press).

With respect to stringency conditions for
30 hybridization, it is well known in the art that numerous

equivalent conditions can be employed to establish a particular stringency by varying, for example, the following factors: the length and nature of probe and target sequences, base composition of the various sequences, concentrations of salts and other hybridization solution components, the presence or absence of blocking agents in the hybridization solutions (e.g., formamide, dextran sulfate, and polyethylene glycol), hybridization reaction temperature and time parameters, as well as, varying wash conditions. The selection of a particular set of hybridization conditions is selected following standard methods in the art (see, for example, Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, (1989) Cold Spring Harbor, N.Y.).

A first polynucleotide is "derived from" second polynucleotide if it has the same or substantially the same basepair sequence as a region of the second polynucleotide, its cDNA, complements thereof, or if it displays sequence identity as described above.

A first polypeptide is "derived from" a second polypeptide if it is (i) encoded by a first polynucleotide derived from a second polynucleotide, or (ii) displays sequence identity to the second polypeptides as described above.

Generally, a viral polypeptide is "derived from" a particular polypeptide of a virus (viral polypeptide) if it is (i) encoded by an open reading frame of a polynucleotide of that virus (viral polynucleotide), or (ii) displays sequence identity to polypeptides of that virus as described above.

"Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 to 5 amino acids, more preferably at least 8 to 10 amino acids, and even more preferably at least 15 to 20 amino acids from a polypeptide encoded by the nucleic acid sequence. Also encompassed are polypeptide sequences which are immunologically identifiable with a polypeptide encoded by the sequence.

"Purified polynucleotide" refers to a polynucleotide of interest or fragment thereof which is essentially free, e.g., contains less than about 50%, preferably less than about 70%, and more preferably less than about 90%, of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity chromatography and sedimentation according to density.

By "nucleic acid immunization" is meant the introduction of a nucleic acid molecule encoding one or more selected antigens into a host cell, for the *in vivo* expression of an antigen, antigens, an epitope, or epitopes. The nucleic acid molecule can be introduced directly into a recipient subject, such as by injection, inhalation, oral, intranasal and mucosal administration, or the like, or can be introduced *ex vivo*, into cells which have been removed from the host. In the latter case, the transformed cells are reintroduced into the subject where an immune response

can be mounted against the antigen encoded by the nucleic acid molecule.

"Gene transfer" or "gene delivery" refers to methods or systems for reliably inserting DNA or RNA of interest into a host cell. Such methods can result in transient expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (e.g., episomes), or integration of transferred genetic material into the genomic DNA of host cells. Gene delivery expression vectors include, but are not limited to, vectors derived from bacterial plasmid vectors, viral vectors, non-viral vectors, alphaviruses, pox viruses and vaccinia viruses. When used for immunization, such gene delivery expression vectors may be referred to as vaccines or vaccine vectors.

"T lymphocytes" or "T cells" are non-antibody producing lymphocytes that constitute a part of the cell-mediated arm of the immune system. T cells arise from immature lymphocytes that migrate from the bone marrow to the thymus, where they undergo a maturation process under the direction of thymic hormones. Here, the mature lymphocytes rapidly divide increasing to very large numbers. The maturing T cells become immunocompetent based on their ability to recognize and bind a specific antigen. Activation of immunocompetent T cells is triggered when an antigen binds to the lymphocyte's surface receptors.

The term "transfection" is used to refer to the uptake of foreign DNA by a cell. A cell has been "transfected" when exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. See, e.g., Graham et al. (1973) *Virology*,

52:456, Sambrook et al. (1989) *Molecular Cloning*, a laboratory manual, Cold Spring Harbor Laboratories, New York, Davis et al. (1986) *Basic Methods in Molecular Biology*, Elsevier, and Chu et al. (1981) *Gene* 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells. The term refers to both stable and transient uptake of the genetic material, and includes uptake of peptide- or antibody-linked DNAs.

A "vector" is capable of transferring gene sequences to target cells (e.g., bacterial plasmid vectors, viral vectors, non-viral vectors, particulate carriers, and liposomes). Typically, "vector construct," "expression vector," and "gene transfer vector," mean any nucleic acid construct capable of directing the expression of a gene of interest and which can transfer gene sequences to target cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

Transfer of a "suicide gene" (e.g., a drug-susceptibility gene) to a target cell renders the cell sensitive to compounds or compositions that are relatively nontoxic to normal cells. Moolten, F.L. (1994) *Cancer Gene Ther.* 1:279-287. Examples of suicide genes are thymidine kinase of herpes simplex virus (HSV-tk), cytochrome P450 (Manome et al. (1996) *Gene Therapy* 3:513-520), human deoxycytidine kinase (Manome et al. (1996) *Nature Medicine* 2(5):567-573) and the bacterial enzyme cytosine deaminase (Dong et al. (1996) *Human Gene Therapy* 7:713-720). Cells which express these genes are rendered sensitive to the effects of the relatively nontoxic prodrugs ganciclovir (HSV-tk), cyclophosphamide (cytochrome P450 2B1), cytosine arabinoside (human deoxycytidine kinase) or 5-fluorocytosine

(bacterial cytosine deaminase). Culver et al. (1992)
Science 256:1550-1552, Huber et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:8302-8306.

5 A "selectable marker" or "reporter marker" refers to a nucleotide sequence included in a gene transfer vector that has no therapeutic activity, but rather is included to allow for simpler preparation, manufacturing, characterization or testing of the gene transfer vector.

10 A "specific binding agent" refers to a member of a specific binding pair of molecules wherein one of the molecules specifically binds to the second molecule through chemical and/or physical means. One example of a specific binding agent is an antibody directed against a selected antigen.

15 By "subject" is meant any member of the subphylum chordata, including, without limitation, humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such
20 as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like. The term does not denote a particular age. Thus, both adult and
25 newborn individuals are intended to be covered. The system described above is intended for use in any of the above vertebrate species, since the immune systems of all of these vertebrates operate similarly.

By "pharmaceutically acceptable" or
30 "pharmacologically acceptable" is meant a material which is not biologically or otherwise undesirable, i.e., the

material may be administered to an individual in a formulation or composition without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is
5 contained.

By "physiological pH" or a "pH in the physiological range" is meant a pH in the range of approximately 7.2 to 8.0 inclusive, more typically in the range of approximately 7.2 to 7.6 inclusive.

10 As used herein, "treatment" refers to any of (i) the prevention of infection or reinfection, as in a traditional vaccine, (ii) the reduction or elimination of symptoms, and (iii) the substantial or complete elimination of the pathogen in question. Treatment may be effected
15 prophylactically (prior to infection) or therapeutically (following infection).

"Lentiviral vector", and "recombinant lentiviral vector" are derived from the subset of retroviral vectors known as lentiviruses. Lentiviral vectors refer to a
20 nucleic acid construct which carries, and within certain embodiments, is capable of directing the expression of a nucleic acid molecule of interest. The lentiviral vector includes at least one transcriptional promoter/enhancer or locus defining element(s), or other elements which control
25 gene expression by other means such as alternate splicing, nuclear RNA export, post-translational modification of messenger, or post-transcriptional modification of protein. Such vector constructs must also include a packaging signal, long terminal repeats (LTRS) or portion thereof, and
30 positive and negative strand primer binding sites appropriate to the lentiviral vector used (if these are not already present in the retroviral vector). Optionally, the

recombinant lentiviral vector may also include a signal which directs polyadenylation, selectable markers such as Neo, TK, hygromycin, phleomycin, histidinol, or DHFR, as well as one or more restriction sites and a translation
5 termination sequence. By way of example, such vectors typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis, and a 3'LTR or a portion thereof.

"Lentiviral vector particle" as utilized within the
10 present invention refers to a lentivirus which carries at least one gene of interest. The retrovirus may also contain a selectable marker. The recombinant lentivirus is capable of reverse transcribing its genetic material (RNA) into DNA and incorporating this genetic material into a host cell's
15 DNA upon infection. Lentiviral vector particles may have a lentiviral envelope, a non-lentiviral envelope (e.g., an amphi or VSV-G envelope), or a chimeric envelope.

"Nucleic acid expression vector" or "Expression
cassette" refers to an assembly which is capable of
20 directing the expression of a sequence or gene of interest. The nucleic acid expression vector includes a promoter which is operably linked to the sequences or gene(s) of interest. Other control elements may be present as well. Expression
cassettes described herein may be contained within a plasmid
25 construct. In addition to the components of the expression cassette, the plasmid construct may also include a bacterial origin of replication, one or more selectable markers, a signal which allows the plasmid construct to exist as
single-stranded DNA (e.g., a M13 origin of replication), a
30 multiple cloning site, and a "mammalian" origin of

replication (e.g., a SV40 or adenovirus origin of replication).

"Packaging cell" refers to a cell which contains those elements necessary for production of infectious recombinant retrovirus (e.g., lentivirus) which are lacking in a recombinant retroviral vector. Typically, such packaging cells contain one or more expression cassettes which are capable of expressing proteins which encode *Gag*, *pol* and *env* proteins.

"Producer cell" or "vector producing cell" refers to a cell which contains all elements necessary for production of recombinant retroviral vector particles.

2. MODES OF CARRYING OUT THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

2.1 SYNTHETIC EXPRESSION CASSETTES

2.1.1 MODIFICATION OF HIV-1 GAG NUCLEIC ACID CODING SEQUENCES

One aspect of the present invention is the generation of HIV-1 Gag protein coding sequences, and related

sequences, having improved expression relative to the corresponding wild-type sequence. An exemplary embodiment of the present invention is illustrated herein modifying the Gag protein wild-type sequences obtained from the HIV-1SF2 strain (SEQ ID NO:1; Sanchez-Pescador, R., et al., *Science* 227(4686): 484-492, 1985; Luciw, P.A., et al. U.S. Patent No. 5,156,949, issued October 20, 1992, herein incorporated by reference; Luciw, P.A., et al., U.S. Patent No. 5,688,688, November 18, 1997, herein incorporated by reference). Gag sequence obtained from other HIV variants may be manipulated in similar fashion following the teachings of the present specification. Such other variants include, but are not limited to, Gag protein encoding sequences obtained from the isolates HIV_{IIIB}, HIV_{SF2}, HIV-1_{SF162}, HIV-1_{SF170}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}, HIV-1_{CM235}, HIV-1_{US4}, other HIV-1 strains from diverse subtypes (e.g., subtypes, A through G, and O), HIV-2 strains and diverse subtypes (e.g., HIV-2_{UC1} and HIV-2_{UC2}), and simian immunodeficiency virus (SIV). (See, e.g., *Virology*, 3rd Edition (W.K. Joklik ed. 1988); *Fundamental Virology*, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991); *Virology*, 3rd Edition (Fields, BN, DM Knipe, PM Howley, Editors, 1996, Lippincott-Raven, Philadelphia, PA; for a description of these and other related viruses).

First, the HIV-1 codon usage pattern was modified so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes (Example 1). The HIV codon usage reflects a high content of the nucleotides A or T of the codon-triplet. The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a decreased translation

ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Gag coding sequences were modified to be comparable to codon usage found in highly expressed human genes. In Figure 11 (Example 1), the percent A-T content of cDNA sequences corresponding to the mRNA for a known unstable mRNA and a known stable mRNA are compared to the percent A-T content of native HIV-1SF2 Gag cDNA and to the synthetic Gag cDNA sequence of the present invention. Experiments performed in support of the present invention showed that the synthetic Gag sequences were capable of higher level of protein production (see the Examples) relative to the native Gag sequences. The data in Figure 11 suggest that one reason for this increased production is increased stability of the mRNA corresponding to the synthetic Gag coding sequences versus the mRNA corresponding to the native Gag coding sequences.

Second, there are inhibitory (or instability) elements (INS) located within the coding sequences of the Gag coding sequences (Example 1). The RRE is a secondary RNA structure that interacts with the HIV encoded Rev-protein to overcome the expression down-regulating effects of the INS. To overcome the post-transcriptional activating mechanisms of RRE and Rev, the instability elements were inactivated by introducing multiple point mutations that did not alter the reading frame of the encoded proteins. Figure 1 shows the original SF2 Gag sequence, the location of the INS sequences, and the modifications made to the INS sequences to reduce their effects. The resulting modified coding sequences are presented as a synthetic Gag expression cassette (SEQ ID NO:4).

Modification of the Gag polypeptide coding sequences resulted in improved expression relative to the wild-type coding sequences in a number of mammalian cell lines (as well as other types of cell lines, including, but not limited to, insect cells). Further, expression of the sequences resulted in production of virus-like particles (VLPs) by these cell lines (see below). Similar Gag polypeptide coding sequences can be obtained from a variety of isolates (families, sub-types, strains, etc.) including, but not limited to such other variants include, but are not limited to, Gag polypeptide encoding sequences obtained from the isolates HIV_{IIIB}, HIV_{SF2}, HIV-1_{SF162}, HIV-1_{SF170}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}, HIV-1_{CM235}, HIV-1_{US4}, other HIV-1 strains from diverse subtypes (e.g., subtypes, A through G, and O), HIV-2 strains and diverse subtypes (e.g., HIV-2_{UC1} and HIV-2_{UC2}), and simian immunodeficiency virus (SIV). (See, e.g., *Virology*, 3rd Edition (W.K. Joklik ed. 1988); *Fundamental Virology*, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991; *Virology*, 3rd Edition (Fields, BN, DM Knipe, PM Howley, Editors, 1996, Lippincott-Raven, Philadelphia, PA). Gag polypeptide encoding sequences derived from these variants can be optimized and tested for improved expression in mammals by following the teachings of the present specification (see the Examples, in particular Example 1).

2.1.2 FURTHER MODIFICATION OF SEQUENCES INCLUDING HIV-1 GAG NUCLEIC ACID CODING SEQUENCES

Experiments performed in support of the present invention have shown that similar modifications of HIV-1 Gag-protease, Gag-reverse transcriptase and Gag-polymerase sequences also result in improved expression of the

polyproteins, as well as, the production of VLPs formed by polypeptides produced from such modified coding sequences.

For the Gag-protease sequence (wild type, SEQ ID NO:2; modified, SEQ ID NOS:5, 78, 79), the changes in codon usage were restricted to the regions upstream of the -1 frameshift (Figure 2). Further, inhibitory (or instability) elements (INS) located within the coding sequences of the Gag-protease polypeptide coding sequence were altered as well (indicated in Figure 2). Exemplary constructs (which include the -1 frameshift) encoding modified Gag-protease sequences include those shown in SEQ ID NOS:78 and 79 (Figures 69 and 70). These are: GP1 (SEQ ID NO:78) in which the protease region was also codon optimized and INS inactivated and GP2 (SEQ ID NO:79), in which the protease region was only subjected to INS inactivation.

For other Gag-containing sequences, for example the Gag-polymerase sequence (wild type, SEQ ID NO:3; modified, SEQ ID NO:6) or Gag-reverse transcriptase (wild type, SEQ ID NO:77; modified SEQ ID NOS:80-84), the changes in codon usage are similar to those for the Gag-protease sequence. Those expression cassettes which contain a frameshift in the GagPol coding sequence are designated "FS(+)" (SEQ ID NOS:80 and 81, Figures 71 and 72) while the designation "FS(-)" (SEQ ID Nos: 82, 83 and 84, Figures 73, 74 and 75) indicates that there is no frameshift utilized in this coding sequence.

In addition to polyproteins containing HIV-related sequences, the various Gag-, Gag-prot, Gag-pol, Gag-reverse transcriptase encoding sequences of the present invention can be fused to other polypeptides (creating chimeric polypeptides) for which an immunogenic response is desired. An example of such a chimeric protein is the joining of the

improved expression Gag encoding sequences to the Hepatitis C Virus (HCV) core protein. In this case, the HCV-core encoding sequences were placed in-frame with the HIV-Gag encoding sequences, resulting in the Gag/HCV-core encoding sequence presented as SEQ ID NO:7 (wild type sequence presented as SEQ ID NO:8).

Further sequences useful in the practice of the present invention include, but are not limited to, sequences encoding viral epitopes/antigens {including but not limited to, HCV antigens (e.g., E1, E2; Houghton, M., et al., U.S. Patent No. 5,714,596, issued February 3, 1998; Houghton, M., et al., U.S. Patent No. 5,712,088, issued January 27, 1998; Houghton, M., et al., U.S. Patent No. 5,683,864, issued November 4, 1997; Weiner, A.J., et al., U.S. Patent No. 5,728,520, issued March 17, 1998; Weiner, A.J., et al., U.S. Patent No. 5,766,845, issued June 16, 1998; Weiner, A.J., et al., U.S. Patent No. 5,670,152, issued September 23, 1997; all herein incorporated by reference), HIV antigens (e.g., derived from *nef*, *tat*, *rev*, *vpu*, *vif*, *vpr* and/or *env*); and sequences encoding tumor antigens/epitopes. Additional sequences are described below. Also, variations on the orientation of the Gag and other coding sequences, relative to each other, are also described below.

Gag, Gag-protease, Gag-reverse transcriptase and/or Gag-polymerase polypeptide coding sequences can be obtained from any HIV isolates (different families, subtypes, and strains) including but not limited to the isolates HIV_{IIIB}, HIV_{SF2}, HIV_{SF162}, HIV_{us4}, HIV_{cm235}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}) (see, e.g., Myers et al. Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico (1992); Myers et al., *Human Retroviruses and Aids*, 1997, Los Alamos, New Mexico:

Los Alamos National Laboratory). Synthetic expression cassettes can be generated using such coding sequences as starting material by following the teachings of the present specification (e.g., see Example 1). Further, the synthetic expression cassettes of the present invention include related Gag polypeptide coding sequences having greater than 75%, preferably greater than 80-85%, more preferably greater than 90-95%, and most preferably greater than 98% sequence identity (or any integer value within these ranges) to the synthetic expression cassette sequences disclosed herein (for example, SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; and SEQ ID NO:20, the Gag Major Homology Region).

2.1.3 EXPRESSION OF SYNTHETIC SEQUENCES ENCODING HIV-1 GAG AND RELATED POLYPEPTIDES

Several synthetic Gag-encoding sequences (expression cassettes) of the present invention were cloned into a number of different expression vectors (Example 1) to evaluate levels of expression and production of VLPs. Two modified synthetic coding sequences are presented as a synthetic Gag expression cassette (SEQ ID NO:4) and a synthetic Gag-protease expression cassette (SEQ ID NOs:78 and 79). Other synthetic Gag-encoding proteins are presented, for example, as SEQ ID NOs:80 through 84. The synthetic DNA fragments for Gag-encoding polypeptides (e.g., Gag, Gag-protease, Gag-polymerase, Gag-reverse transcriptase) were cloned into expression vectors described in Example 1, including, a transient expression vector, CMV-promoter-based mammalian vectors, and a shuttle vector for use in baculovirus expression systems. Corresponding wild-type sequences were cloned into the same vectors.

These vectors were then transfected into a several different cell types, including a variety of mammalian cell lines, (293, RD, COS-7, and CHO, cell lines available, for example, from the A.T.C.C.). The cell lines were cultured under appropriate conditions and the levels of p24 (Gag) expression in supernatants were evaluated (Example 2). The results of these assays demonstrated that expression of synthetic Gag-encoding sequences were significantly higher than corresponding wild-type sequences (Example 2; Table 2).

Further, Western Blot analysis showed that cells containing the synthetic Gag expression cassette produced the expected 55 kD (p55) protein at higher per-cell concentrations than cells containing the native expression cassette. The Gag p55 protein was seen in both cell lysates and supernatants. The levels of production were significantly higher in cell supernatants for cells transfected with the synthetic Gag expression cassette of the present invention. Experiments performed in support of the present invention suggest that cells containing the synthetic Gag-prot expression cassettes produced the expected Gag-prot protein at comparably higher per-cell concentrations than cells containing the wild-type expression cassette.

Fractionation of the supernatants from mammalian cells transfected with the synthetic Gag expression cassette showed that it provides superior production of both p55 protein and VLPs, relative to the wild-type Gag sequences (Examples 6 and 7).

Efficient expression of these Gag-containing polypeptides in mammalian cell lines provides the following benefits: the Gag polypeptides are free of baculovirus contaminants; production by established methods approved by

the FDA; increased purity; greater yields (relative to native coding sequences); and a novel method of producing the Gag-containing polypeptides in CHO or other mammalian cells which is not feasible in the absence of the increased expression obtained using the constructs of the present invention. Exemplary Mammalian cell lines include, but are not limited to, BHK, VERO, HT1080, 293, 293T, RD, COS-7, CHO, Jurkat, HUT, SUPT, C8166, MOLT4/clone8, MT-2, MT-4, H9, PM1, CEM, myeloma cells (e.g., SB20 cells) and CEMX174, such cell lines are available, for example, from the A.T.C.C.).

A synthetic Gag expression cassette of the present invention also demonstrated high levels of expression and VLP production when transfected into insect cells (Example 7). Further, in addition to a higher total protein yield, the final product from the synthetic p55-expressed Gag consistently contained lower amounts of contaminating baculovirus proteins than the final purified product from the native p55-expressed Gag.

Further, synthetic Gag expression cassettes of the present invention have also been introduced into yeast vectors which were transformed into and efficiently expressed by yeast cells (*Saccharomyces cerevisiae*; using vectors as described in Rosenberg, S. and Tekamp-Olson, P., U.S. Patent No. RE35,749, issued, March 17, 1998, herein incorporated by reference).

In addition to the mammalian and insect vectors described in the Examples, the synthetic expression cassettes of the present invention can be incorporated into a variety of expression vectors using selected expression control elements. Appropriate vectors and control elements for any given cell type can be selected by one having

ordinary skill in the art in view of the teachings of the present specification and information known in the art about expression vectors.

For example, a synthetic Gag expression cassette can be
5 inserted into a vector which includes control elements operably linked to the desired coding sequence, which allow for the expression of the gene in a selected cell-type. For example, typical promoters for mammalian cell expression include the SV40 early promoter, a CMV promoter such as the
10 CMV immediate early promoter (a CMV promoter can include intron A), RSV, HIV-LTR, the mouse mammary tumor virus LTR promoter (MMLV-LTR), FIV-LTR, the adenovirus major late promoter (Ad MLP), and the herpes simplex virus promoter, among others. Other nonviral promoters, such as a promoter
15 derived from the murine metallothionein gene, will also find use for mammalian expression. Typically, transcription termination and polyadenylation sequences will also be present, located 3' to the translation stop codon. Preferably, a sequence for optimization of initiation of
20 translation, located 5' to the coding sequence, is also present. Examples of transcription terminator/polyadenylation signals include those derived from SV40, as described in Sambrook, et al., *supra*, as well as a bovine growth hormone terminator sequence. Introns,
25 containing splice donor and acceptor sites, may also be designed into the constructs for use with the present invention (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986).

Enhancer elements may also be used herein to increase
30 expression levels of the mammalian constructs. Examples include the SV40 early gene enhancer, as described in

Dijkema et al., *EMBO J.* (1985) 4:761, the enhancer/promoter derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus, as described in Gorman et al., *Proc. Natl. Acad. Sci. USA* (1982b) 79:6777 and elements derived from human CMV, as described in Boshart et al., *Cell* (1985) 41:521, such as elements included in the CMV intron A sequence (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986).

The desired synthetic Gag polypeptide encoding sequences can be cloned into any number of commercially available vectors to generate expression of the polypeptide in an appropriate host system. These systems include, but are not limited to, the following: baculovirus expression {Reilly, P.R., et al., BACULOVIRUS EXPRESSION VECTORS: A LABORATORY MANUAL (1992); Beames, et al., *Biotechniques* 11:378 (1991); Pharmingen; Clontech, Palo Alto, CA}}, vaccinia expression {Earl, P. L., et al., "Expression of proteins in mammalian cells using vaccinia" In *Current Protocols in Molecular Biology* (F. M. Ausubel, et al. Eds.), Greene Publishing Associates & Wiley Interscience, New York (1991); Moss, B., et al., U.S. Patent Number 5,135,855, issued 4 August 1992}, expression in bacteria {Ausubel, F.M., et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, Inc., Media PA; Clontech}, expression in yeast {Rosenberg, S. and Tekamp-Olson, P., U.S. Patent No. RE35,749, issued, March 17, 1998, herein incorporated by reference; Shuster, J.R., U.S. Patent No. 5,629,203, issued May 13, 1997, herein incorporated by reference; Gellissen, G., et al., *Antonie Van Leeuwenhoek*, 62(1-2):79-93 (1992); Romanos, M.A., et al., *Yeast* 8(6):423-488 (1992); Goeddel, D.V., *Methods in Enzymology* 185 (1990); Guthrie, C., and G.R. Fink, *Methods*

in *Enzymology* 194 (1991)}, expression in mammalian cells {Clontech; Gibco-BRL, Ground Island, NY; e.g., Chinese hamster ovary (CHO) cell lines (Haynes, J., et al., *Nuc. Acid. Res.* 11:687-706 (1983); 1983, Lau, Y.F., et al., *Mol. Cell. Biol.* 4:1469-1475 (1984); Kaufman, R. J., "Selection and coamplification of heterologous genes in mammalian cells," in *Methods in Enzymology*, vol. 185, pp537-566. Academic Press, Inc., San Diego CA (1991)}, and expression in plant cells {plant cloning vectors, Clontech Laboratories, Inc., Palo Alto, CA, and Pharmacia LKB Biotechnology, Inc., Piscataway, NJ; Hood, E., et al., *J. Bacteriol.* 168:1291-1301 (1986); Nagel, R., et al., *FEMS Microbiol. Lett.* 67:325 (1990); An, et al., "Binary Vectors", and others in *Plant Molecular Biology Manual* A3:1-19 (1988); Miki, B.L.A., et al., pp.249-265, and others in *Plant DNA Infectious Agents* (Hohn, T., et al., eds.) Springer-Verlag, Wien, Austria, (1987); *Plant Molecular Biology: Essential Techniques*, P.G. Jones and J.M. Sutton, New York, J. Wiley, 1997; Miglani, Gurbachan *Dictionary of Plant Genetics and Molecular Biology*, New York, Food Products Press, 1998; Henry, R. J., *Practical Applications of Plant Molecular Biology*, New York, Chapman & Hall, 1997}.

Also included in the invention is an expression vector, such as the CMV promoter-containing vectors described in Example 1, containing coding sequences and expression control elements which allow expression of the coding regions in a suitable host. The control elements generally include a promoter, translation initiation codon, and translation and transcription termination sequences, and an insertion site for introducing the insert into the vector. Translational control elements have been reviewed by M.

Kozak (e.g., Kozak, M., *Mamm. Genome* 7(8):563-574, 1996; Kozak, M., *Biochimie* 76(9):815-821, 1994; Kozak, M., *J Cell Biol* 108(2):229-241, 1989; Kozak, M., and Shatkin, A.J., *Methods Enzymol* 60:360-375, 1979).

5 Expression in yeast systems has the advantage of commercial production. Recombinant protein production by vaccinia and CHO cell line have the advantage of being mammalian expression systems. Further, vaccinia virus expression has several advantages including the following:
10 (i) its wide host range; (ii) faithful post-transcriptional modification, processing, folding, transport, secretion, and assembly of recombinant proteins; (iii) high level expression of relatively soluble recombinant proteins; and (iv) a large capacity to accommodate foreign DNA.

15 The recombinantly expressed polypeptides from synthetic Gag-encoding expression cassettes are typically isolated from lysed cells or culture media. Purification can be carried out by methods known in the art including salt fractionation, ion exchange chromatography, gel filtration, size-exclusion chromatography, size-fractionation, and
20 affinity chromatography. Immunoaffinity chromatography can be employed using antibodies generated based on, for example, Gag antigens.

Advantages of expressing the Gag-containing proteins of
25 the present invention using mammalian cells include, but are not limited to, the following: well-established protocols for scale-up production; the ability to produce VLPs; cell lines are suitable to meet good manufacturing process (GMP) standards; culture conditions for mammalian cells are known
30 in the art.

2.1.4 MODIFICATION OF HIV-1 ENV NUCLEIC ACID CODING
SEQUENCES

One aspect of the present invention is the generation of HIV-1 Env protein coding sequences, and related sequences, having improved expression relative to the corresponding wild-type sequence. Exemplary embodiments of the present invention are illustrated herein modifying the Env protein wild-type sequences obtained from the HIV-1 subtype B strains HIV-1US4 and HIV-1SF162 (Myers et al., Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico (1992); Myers et al., *Human Retroviruses and Aids*, 1997, Los Alamos, New Mexico: Los Alamos National Laboratory). Env sequence obtained from other HIV variants may be manipulated in similar fashion following the teachings of the present specification. Such other variants include those described above in Section 2.1.1 and on the World Wide Web (Internet), for example at <http://hiv-web.lanl.gov/cgi-bin/hivDB3/public/wdb/ssampublic> and <http://hiv-web.lanl.gov>.

First, the HIV-1 codon usage pattern was modified so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes (Example 1). The HIV codon usage reflects a high content of the nucleotides A or T of the codon-triplet. The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Env coding sequences were modified to be comparable to codon usage found in highly expressed human genes. Experiments performed in support of the present invention showed that

the synthetic Env sequences were capable of higher level of protein production (see the Examples) relative to the native Env sequences. One reason for this increased production may be increased stability of the mRNA corresponding to the synthetic Env coding sequences versus the mRNA corresponding to the native Env coding sequences.

Modification of the Env polypeptide coding sequences resulted in improved expression relative to the wild-type coding sequences in a number of mammalian cell lines.

Similar Env polypeptide coding sequences can be obtained from a variety of isolates (families, sub-types, etc.). Env polypeptide encoding sequences derived from these variants can be optimized and tested for improved expression in mammals by following the teachings of the present specification (see the Examples, in particular Example 2).

2.1.5 FURTHER MODIFICATION OF HIV-1 ENV NUCLEIC ACID CODING SEQUENCES

In addition to proteins containing HIV-related sequences, the Env encoding sequences of the present invention can be fused to other polypeptides (creating chimeric polypeptides). Also, variations on the orientation of the Env and other coding sequences, relative to each other, are contemplated. Further, the HIV protein encoding cassettes of the present invention can be co-expressed using one vector or multiple vectors. In addition, the polyproteins can be operably linked to the same or different promoters.

Env polypeptide coding sequences can be obtained from any HIV isolates (different families, subtypes, and strains) including but not limited to the isolates HIV_{IIIB}, HIV_{SF2},

HIV_{us4}, HIV_{CM235}, HIV_{SF162}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}) (see, e.g., Myers et al., Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico (1992); Myers et al., *Human Retroviruses and Aids*, 1997, Los Alamos, New Mexico: Los Alamos National Laboratory). Synthetic expression cassettes can be generated using such coding sequences as starting material by following the teachings of the present specification (e.g., see Example 1). Further, the synthetic expression cassettes (and purified polynucleotides) of the present invention include related Env polypeptide coding sequences having greater than 90%, preferably greater than 92%, more preferably greater than 95%, and most preferably greater than 98% sequence identity to the synthetic expression cassette sequences disclosed herein (for example, SEQ ID NOs:71-72; and/or the sequences presented in Tables 1A and 1B) when the sequences of the present invention are used as the query sequence.

2.1.6 EXPRESSION OF SYNTHETIC SEQUENCES ENCODING HIV-1 ENV AND RELATED POLYPEPTIDES

Several synthetic Env-encoding sequences (expression cassettes) of the present invention were cloned into a number of different expression vectors (Example 1) to evaluate levels of expression and production of Env polypeptide. A modified synthetic coding sequence is presented as synthetic Env expression cassettes (Example 1, e.g., Tables 1A and 1B). The synthetic DNA fragments for Env were cloned into eucaryotic expression vectors described in Example 1 and in Section 2.1.3 above, including, a transient expression vector and CMV-promoter-based mammalian

vectors. Corresponding wild-type sequences were cloned into the same vectors.

These vectors were then transfected into a several different cell types, including a variety of mammalian cell lines, (293, RD, COS-7, and CHO, cell lines available, for example, from the A.T.C.C.). The cell lines were cultured under appropriate conditions and the levels of gp120, gp140 and gp160 Env expression in supernatants were evaluated (Example 2). Env polypeptides include, but are not limited to, for example, native gp160, oligomeric gp140, monomeric gp120 as well as modified sequences of these polypeptides. The results of these assays demonstrated that expression of synthetic Env encoding sequences were significantly higher than corresponding wild-type sequences (Example 2; Tables 3 and 4).

Further, Western Blot analysis showed that cells containing the synthetic Env expression cassette produced the expected protein (gp120, gp140 or gp160) at higher per-cell concentrations than cells containing the native expression cassette. The Env proteins were seen in both cell lysates and supernatants. The levels of production were significantly higher in cell supernatants for cells transfected with the synthetic Env expression cassettes of the present invention as compared to wild type.

Fractionation of the supernatants from mammalian cells transfected with the synthetic Env expression cassettes showed that it provides superior production of Env proteins, relative to the wild-type Env sequences (Examples 2 and 3).

Efficient expression of these Env-containing polypeptides in mammalian cell lines provides the following benefits: the Env polypeptides are free of baculovirus or other viral contaminants; production by established methods

approved by the FDA; increased purity; greater yields
(relative to native coding sequences); and a novel method of
producing the Env-containing polypeptides in CHO cells which
is less feasible in the absence of the increased expression
5 obtained using the constructs of the present invention.

Exemplary cell lines (e.g., mammalian, yeast, insect,
etc.) include those described above in Section 2.1.3 for
Gag-containing constructs. Further, appropriate vectors and
control elements (e.g., promoters, enhancers,
10 polyadenylation sequences, etc.) for any given cell type can
be selected, as described above in Section 2.1.3, by one
having ordinary skill in the art in view of the teachings of
the present specification and information known in the art
about expression vectors. In addition, the recombinantly
15 expressed polypeptides from synthetic Env-encoding
expression cassettes are typically isolated and purified
from lysed cells or culture media, as described above for
Gag-encoding expression cassettes. An exemplary
purification is described in Example 4 and shown in Figure
20 60.

2.1.7 MODIFICATION OF HIV-1 TAT NUCLEIC ACID CODING SEQUENCES

Another aspect of the present invention is the
25 generation of HIV-1 tat protein coding sequences, and
related sequences, having improved expression relative to
the corresponding wild-type sequence. Exemplary embodiments
of the present invention are illustrated herein modifying
the tat wild-type nucleotide sequence (SEQ ID NO:85, Figure
30 76) obtained from SF162 as described above. Exemplary
synthetic tat constructs are shown in SEQ ID NO:87, which

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depicts a tat construct encoding a full-length tat polypeptide from strain SF162; SEQ ID NO:88, which depicts a tat construct encoding a tat polypeptide having the cystein residue at position 22 changed; and SEQ ID NO:89, which

5 depicts a tat construct encoding the amino terminal portion of a tat polypeptide from strain SF162. The amino portion of the tat protein appears to contain many of the epitopes that induce an immune response. In addition, further modifications include replacement or deletion of the cystein

10 residue at position 22, for example with a valine residue, an alanine residue or a glycine residue (SEQ ID Nos: 88 and 89, Figures 79 and 81), see, e.g., Caputo et al. (1996) *Gene Ther.* 3:235. In Figure 81, which depicts a tat construct encoding the amino terminal portion of a tat polypeptide,

15 the nucleotides (nucleotides 64-66) encoding the cystein residues are underlined. The design and construction of suitable construct can be readily done using the teachings of the present specification. As with Gag, pol, prot and Env, tat polypeptide coding sequences can be

20 obtained from a variety of isolates (families, sub-types, etc.).

Modification of the tat polypeptide coding sequences result in improved expression relative to the wild-type coding sequences in a number of cell lines (e.g., mammalian,

25 yeast, bacterial and insect cells). Tat polypeptide encoding sequences derived from these variants can be optimized and tested for improved expression in mammals by following the teachings of the present specification (see the Examples, in particular Example 2).

30 Various forms of the different embodiments of the invention, described herein, may be combined. For example,

polynucleotides may be derived from the polynucleotide sequences of the present invention, including, but not limited to, coding sequences for Gag polypeptides, Env polypeptides, polymerase polypeptides, protease polypeptides, tat polypeptides, and reverse transcriptase polypeptides. Further, the polynucleotide coding sequences of the present invention may be combined into multicistronic expression cassettes where typically each coding sequence for each polypeptide is preceded by IRES sequences.

2.2 PRODUCTION OF VIRUS-LIKE PARTICLES AND USE OF THE CONSTRUCTS OF THE PRESENT INVENTION TO CREATE PACKAGING CELL LINES

The group-specific antigens (Gag) of human immunodeficiency virus type-1 (HIV-1) self-assemble into noninfectious virus-like particles (VLP) that are released from various eucaryotic cells by budding (reviewed by Freed, E.O., *Virology* 251:1-15, 1998). The synthetic expression cassettes of the present invention provide efficient means for the production of HIV-Gag virus-like particles (VLPs) using a variety of different cell types, including, but not limited to, mammalian cells.

Viral particles can be used as a matrix for the proper presentation of an antigen entrapped or associated therewith to the immune system of the host. For example, U.S. Patent No. 4,722,840 describes hybrid particles comprised of a particle-forming fragment of a structural protein from a virus, such as a particle-forming fragment of hepatitis B virus (HBV) surface antigen (HBsAg), fused to a heterologous polypeptide. Tindle et al., *Virology* (1994) 200:547-557, describes the production and use of chimeric HBV core

antigen particles containing epitopes of human papillomavirus (HPV) type 16 E7 transforming protein.

Adams et al., *Nature* (1987) 329:68-70, describes the recombinant production of hybrid HIVgp120:Ty VLPs in yeast
5 and Brown et al., *Virology* (1994) 198:477-488, the production of chimeric proteins consisting of the VP2 protein of human parvovirus B19 and epitopes from human herpes simplex virus type 1, as well as mouse hepatitis virus A59. Wagner et al., (*Virology* (1994) 200:162-175,
10 Brand et al., *J. Virol. Meth.* (1995) 51:153-168; *Virology* (1996) 220:128-140) and Wolf, et al., (EP 0 449 116 A1, published 2 October 1991; WO 96/30523, published 3 October 1996) describe the assembly of chimeric HIV-1 p55Gag particles. U.S. Patent No. 5,503,833 describes the use of
15 rotavirus VP6 spheres for encapsulating and delivering therapeutic agents.

2.2.1 VLP PRODUCTION USING THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT INVENTION

20 Experiments performed in support of the present invention have demonstrated that the synthetic expression cassettes of the present invention provide superior production of both protein and VLPs, relative to native coding sequences (Examples 7 and 15). Further, electron
25 microscopic evaluation of VLP production (Examples 6 and 15, Figures 3A-B and 65A-F) showed that free and budding immature virus particles of the expected size were produced by cells containing the synthetic expression cassettes.

Using the synthetic expression cassettes of the present
30 invention, rather than native coding sequences, for the production of virus-like particles provide several

advantages. First, VLPs can be produced in enhanced quantity making isolation and purification of the VLPs easier. Second, VLPs can be produced in a variety of cell types using the synthetic expression cassettes, in particular, mammalian cell lines can be used for VLP production, for example, CHO cells. Production using CHO cells provides (i) VLP formation; (ii) correct myristylation and budding; (iii) absence of non-mammalian cell contaminants (e.g., insect viruses and/or cells); and (iv) ease of purification. The synthetic expression cassettes of the present invention are also useful for enhanced expression in cell-types other than mammalian cell lines. For example, infection of insect cells with baculovirus vectors encoding the synthetic expression cassettes resulted in higher levels of total protein yield and higher levels of VLP production (relative to wild-type coding sequences). Further, the final product from insect cells infected with the baculovirus-Gag synthetic expression cassettes consistently contained lower amounts of contaminating insect proteins than the final product when wild-type coding sequences were used (Examples).

VLPs can spontaneously form when the particle-forming polypeptide of interest is recombinantly expressed in an appropriate host cell. Thus, the VLPs produced using the synthetic expression cassettes of the present invention are conveniently prepared using recombinant techniques. As discussed below, the Gag polypeptide encoding synthetic expression cassettes of the present invention can include other polypeptide coding sequences of interest (for example, Env, tat, rev, HIV protease, HIV polymerase, HCV core; see, Example 1). Expression of such synthetic expression cassettes yields VLPs comprising the product of the

synthetic expression cassette, as well as, the polypeptide of interest.

Once coding sequences for the desired particle-forming polypeptides have been isolated or synthesized, they can be
5 cloned into any suitable vector or replicon for expression. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. See, generally, Ausubel et al, *supra* or Sambrook et al, *supra*. The vector is then used to transform
10 an appropriate host cell. Suitable recombinant expression systems include, but are not limited to, bacterial, mammalian, baculovirus/insect, vaccinia, Semliki Forest virus (SFV), Alphaviruses (such as, Sindbis, Venezuelan Equine Encephalitis (VEE)), mammalian, yeast and *Xenopus*
15 expression systems, well known in the art. Particularly preferred expression systems are mammalian cell lines, vaccinia, Sindbis, insect and yeast systems.

For example, a number of mammalian cell lines are known in the art and include immortalized cell lines available
20 from the American Type Culture Collection (A.T.C.C.), such as, but not limited to, Chinese hamster ovary (CHO) cells, 293 cells, HeLa cells, baby hamster kidney (BHK) cells, mouse myeloma (SB20), monkey kidney cells (COS), as well as others. Similarly, bacterial hosts such as *E. coli*,
25 *Bacillus subtilis*, and *Streptococcus spp.*, will find use with the present expression constructs. Yeast hosts useful in the present invention include *inter alia*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*,
30 *Pichia guillerimondii*, *Pichia pastoris*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*. Insect cells for use with

baculovirus expression vectors include, *inter alia*, *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni*. See, e.g., Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987). Fungal hosts include, for example, *Aspergillus*.

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Viral vectors can be used for the production of particles in eucaryotic cells, such as those derived from the pox family of viruses, including vaccinia virus and avian poxvirus. Additionally, a vaccinia based infection/transfection system, as described in Tomei et al., *J. Virol.* (1993) 67:4017-4026 and Selby et al., *J. Gen. Virol.* (1993) 74:1103-1113, will also find use with the present invention. In this system, cells are first infected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the DNA of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translational machinery. Alternately, T7 can be added as a purified protein or enzyme as in the "Progenitor" system (Studier and Moffatt, *J. Mol. Biol.* (1986) 189:113-130). The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation product(s).

Depending on the expression system and host selected, the VLPS are produced by growing host cells transformed by an expression vector under conditions whereby the particle-

forming polypeptide is expressed and VLPs can be formed. The selection of the appropriate growth conditions is within the skill of the art. If the VLPs are formed intracellularly, the cells are then disrupted, using
5 chemical, physical or mechanical means, which lyse the cells yet keep the VLPs substantially intact. Such methods are known to those of skill in the art and are described in, e.g., *Protein Purification Applications: A Practical Approach*, (E.L.V. Harris and S. Angal, Eds., 1990).

10 The particles are then isolated (or substantially purified) using methods that preserve the integrity thereof, such as, by density gradient centrifugation, e.g., sucrose gradients, PEG-precipitation, pelleting, and the like (see, e.g., Kirnbauer et al. *J. Virol.* (1993) 67:6929-6936), as
15 well as standard purification techniques including, e.g., ion exchange and gel filtration chromatography.

VLPs produced by cells containing the synthetic expression cassettes of the present invention can be used to elicit an immune response when administered to a subject.
20 One advantage of the present invention is that VLPs can be produced by mammalian cells carrying the synthetic expression cassettes at levels previously not possible. As discussed above, the VLPs can comprise a variety of antigens in addition to the Gag polypeptides (e.g., Env, tat, Gag-
25 protease, Gag-polymerase, Gag-HCV-core). Purified VLPs, produced using the synthetic expression cassettes of the present invention, can be administered to a vertebrate subject, usually in the form of vaccine compositions. Combination vaccines may also be used, where such vaccines
30 contain, for example, other subunit proteins derived from HIV or other organisms (e.g., env) or gene delivery vaccines

encoding such antigens. Administration can take place using the VLPs formulated alone or formulated with other antigens. Further, the VLPs can be administered prior to, concurrent with, or subsequent to, delivery of the synthetic expression cassettes for DNA immunization (see below) and/or delivery of other vaccines. Also, the site of VLP administration may be the same or different as other vaccine compositions that are being administered. Gene delivery can be accomplished by a number of methods including, but are not limited to, immunization with DNA, alphavirus vectors, pox virus vectors, and vaccinia virus vectors.

VLP immune-stimulating (or vaccine) compositions can include various excipients, adjuvants, carriers, auxiliary substances, modulating agents, and the like. The immune stimulating compositions will include an amount of the VLP/antigen sufficient to mount an immunological response. An appropriate effective amount can be determined by one of skill in the art. Such an amount will fall in a relatively broad range that can be determined through routine trials and will generally be an amount on the order of about 0.1 μg to about 1000 μg , more preferably about 1 μg to about 300 μg , of VLP/antigen.

A carrier is optionally present which is a molecule that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycollic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived

from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; McGee JP, et al., *J Microencapsul.* 14(2):197-210, 1997; O'Hagan DT, et al., *Vaccine* 11(2):149-54, 1993.

5 Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen may be conjugated to a bacterial toxoid, such as toxoid from diphtheria, tetanus, cholera, etc., as well as
10 toxins derived from *E. coli*.

Such adjuvants include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc.; (2) oil-in-water emulsion formulations (with or without other specific
15 immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (International Publication No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE
20 (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized
25 into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL),
30 trehalose dimycolate (TDM), and cell wall skeleton (CWS),

preferably MPL + CWS (Detox™); (3) saponin adjuvants, such as Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particle generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (IL-1, IL-2, etc.), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), beta chemokines (MIP, 1-alpha, 1-beta Rantes, etc.); (6) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* heat-labile toxin (LT), particularly LT-K63 (where lysine is substituted for the wild-type amino acid at position 63) LT-R72 (where arginine is substituted for the wild-type amino acid at position 72), CT-S109 (where serine is substituted for the wild-type amino acid at position 109), and PT-K9/G129 (where lysine is substituted for the wild-type amino acid at position 9 and glycine substituted at position 129) (see, e.g., International Publication Nos. W093/13202 and W092/19265); and (7) other substances that act as immunostimulating agents to enhance the effectiveness of the composition.

Muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

Dosage treatment with the VLP composition may be a single dose schedule or a multiple dose schedule. A multiple dose schedule is one in which a primary course of

vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals, chosen to maintain and/or reinforce the immune response, for example at 1-4 months for a second dose, and if needed, a subsequent
5 dose(s) after several months. The dosage regimen will also, at least in part, be determined by the potency of the modality, the vaccine delivery employed, the need of the subject and be dependent on the judgment of the practitioner.

10 If prevention of disease is desired (e.g., reduction of symptoms, recurrences or of disease progression), the antigen carrying VLPs are generally administered prior to primary infection with the pathogen of interest. If treatment is desired, e.g., the reduction of symptoms or
15 recurrences, the VLP compositions are generally administered subsequent to primary infection.

2.2.2 USING THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT INVENTION TO CREATE PACKAGING CELL LINES

20 A number of viral based systems have been developed for use as gene transfer vectors for mammalian host cells. For example, retroviruses (in particular, lentiviral vectors) provide a convenient platform for gene delivery systems. A coding sequence of interest (for example, a sequence useful
25 for gene therapy applications) can be inserted into a gene delivery vector and packaged in retroviral particles using techniques known in the art. Recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems have been
30 described, including, for example, the following: (U.S. Patent No. 5,219,740; Miller et al. (1989) *Biotechniques*

7:980; Miller, A.D. (1990) *Human Gene Therapy* 1:5; Scarpa et al. (1991) *Virology* 180:849; Burns et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:8033; Boris-Lawrie et al. (1993) *Cur. Opin. Genet. Develop.* 3:102; GB 2200651; EP 0415731; EP 5 0345242; WO 89/02468; WO 89/05349; WO 89/09271; WO 90/02806; WO 90/07936; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; in U.S. 5,219,740; U.S. 4,405,712; U.S. 4,861,719; U.S. 4,980,289 and U.S. 4,777,127; in U.S. Serial No. 07/800,921; and in 10 Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53:83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci USA* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

15 Sequences useful for gene therapy applications include, but are not limited to, the following. Factor VIII cDNA, including derivatives and deletions thereof (International Publication Nos. WO 96/21035, WO 97/03193, WO 97/03194, WO 97/03195, and WO 97/03191, all of which are hereby 20 incorporated by reference). Factor IX cDNA (Kurachi et al. (1982) *Proc. Natl. Acad. Sci. USA* 79:6461-6464). Factor V cDNA can be obtained from pMT2-V (Jenny (1987) *Proc. Natl. Acad. Sci. USA* 84:4846, A.T.C.C. Deposit No. 40515). A full-length factor V cDNA, or a B domain deletion or B 25 domain substitution thereof, can be used. B domain deletions of factor V, include those reported by Marquette (1995) *Blood* 86:3026 and Kane (1990) *Biochemistry* 29:6762. Antithrombin III cDNA (Prochownik (1983) *J. Biol. Chem.* 258:8389, A.T.C.C. Deposit No. 57224/57225). Protein C 30 encoding cDNA (Foster (1984) *Proc. Natl. Acad. Sci. USA* 81:4766; Beckmann (1985) *Nucleic Acids Res.* 13:5233).

Prothrombin cDNA can be obtained by restriction enzyme digestion of a published vector (Degen (1983) *Biochemistry* 22:2087). The endothelial cell surface protein, thrombomodulin, is a necessary cofactor for the normal
5 activation of protein C by thrombin. A soluble recombinant form has been described (Parkinson (1990) *J. Biol. Chem.* 265:12602; Jackman (1987) *Proc. Natl. Acad. Sci. USA* 84:6425; Shirai (1988) *J. Biochem.* 103:281; Wen (1987) *Biochemistry* 26:4350; Suzuki (1987) *EMBO J.* 6:1891, A.T.C.C.
10 Deposit No. 61348, 61349).

Many genetic diseases caused by inheritance of defective genes result in the failure to produce normal gene products, for example, thalassemia, phenylketonuria, Lesch-Nyhan syndrome, severe combined immunodeficiency (SCID),
15 hemophilia A and B, cystic fibrosis, Duchenne's Muscular Dystrophy, inherited emphysema and familial hypercholesterolemia (Mulligan et al. (1993) *Science* 260:926; Anderson et al. (1992) *Science* 256:808; Friedman et al. (1989) *Science* 244:1275). Although genetic diseases may
20 result in the absence of a gene product, endocrine disorders, such as diabetes and hypopituitarism, are caused by the inability of the gene to produce adequate levels of the appropriate hormone insulin and human growth hormone respectively.

25 In one aspect, gene therapy employing the constructs and methods of the present invention involves the introduction of normal recombinant genes into T cells so that new or missing proteins are produced by the T cells after introduction or reintroduction thereof into a patient.
30 A number of genetic diseases have been selected for treatment with gene therapy, including adenine deaminase

deficiency, cystic fibrosis, α_1 -antitrypsin deficiency, Gaucher's syndrome, as well as non-genetic diseases.

In particular, Gaucher's syndrome is a genetic disorder characterized by a deficiency of the enzyme
5 glucocerebrosidase. This enzyme deficiency leads to the accumulation of glucocerebroside in the lysosomes of all cells in the body. For a review see *Science* 256:794 (1992) and Scriver et al., *The Metabolic Basis of Inherited Disease*, 6th ed., vol. 2, page 1677). Thus, gene transfer
10 vectors that express glucocerebrosidase can be constructed for use in the treatment of this disorder. Likewise, gene transfer vectors encoding lactase can be used in the treatment of hereditary lactose intolerance, those expressing AD can be used for treatment of ADA deficiency,
15 and gene transfer vectors encoding α_1 -antitrypsin can be used to treat α_1 -antitrypsin deficiency. See Ledley, F.D. (1987) *J. Pediatrics* 110:157-174, Verma, I. (Nov. 1987) *Scientific American* pp. 68-84, and International Publication No. WO 95/27512 entitled "Gene Therapy Treatment for a
20 Variety of Diseases and Disorders," for a description of gene therapy treatment of genetic diseases.

In still further embodiments of the invention, nucleotide sequences which can be incorporated into a gene transfer vector include, but are not limited to, proteins
25 associated with enzyme-deficiency disorders, such as the cystic fibrosis transmembrane regulator (see, for example, U.S. Patent No. 5,240,846 and Larrick et al. (1991) *Gene Therapy Applications of Molecular Biology*, Elsevier, New York and adenosine deaminase (ADA) (see U.S. Patent No.
30 5,399,346); growth factors, or an agonist or antagonist of a growth factor (Bandara et al. (1992) *DNA and Cell Biology*,

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11:227); one or more tumor suppressor genes such as p53, Rb, or C-CAMI (Kleinerman et al. (1995) *Cancer Research* 55:2831); a molecule that modulates the immune system of an organism, such as a HLA molecule (Nabel et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:11307); a ribozyme (Larsson et al. (1996) *Virology* 219:161); a peptide nucleic acid (Hirshman et al. (1996) *J. Invest. Med.* 44:347); an antisense molecule (Bordier et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:9383) which can be used to down-regulate the expression or synthesis of aberrant or foreign proteins, such as HIV proteins or a wide variety of oncogenes such as p53 (Hesketh, *The Oncogene Facts Book*, Academic Press, New York, (1995); a biopharmaceutical agent or antisense molecule used to treat HIV-infection, such as an inhibitor of p24 (Nakashima et al. (1994) *Nucleic Acids Res.* 22:5004); or reverse-transcriptase (see, Bordier, *supra*).

Other proteins of therapeutic interest can be expressed *in vivo* by gene transfer vectors using the methods of the invention. For instance sustained *in vivo* expression of tissue factor inhibitory protein (TFPI) is useful for treatment of conditions including sepsis and DIC and in preventing reperfusion injury. (See International Publications Nos. WO 93/24143, WO 93/25230 and WO 96/06637). Nucleic acid sequences encoding various forms of TFPI can be obtained, for example, as described in US Patent Nos. 4,966,852; 5,106,833; and 5,466,783, and incorporated into the gene transfer vectors described herein.

Erythropoietin (EPO) and leptin can also be expressed *in vivo* from genetically modified T cells according to the methods of the invention. For instance EPO is useful in gene therapy treatment of a variety of disorders including

anemia (see International Publication No. WO 95/13376
entitled "Gene Therapy for Treatment of Anemia"). Sustained
delivery of leptin by the methods of the invention is useful
in treatment of obesity. See International Publication No.
5 WO 96/05309 for a description of the leptin gene and the use
thereof in the treatment of obesity.

A variety of other disorders can also be treated by the
methods of the invention. For example, sustained *in vivo*
systemic production of apolipoprotein E or apolipoprotein A
10 from genetically modified T cells can be used for treatment
of hyperlipidemia (see Breslow et al. (1994) *Biotechnology*
12:365). Sustained production of angiotensin receptor
inhibitor (Goodfriend et al. (1996) *N. Engl. J. Med.*
334:1469) can be provided by the methods described herein.
15 As yet an additional example, the long term *in vivo* systemic
production of angiostatin is useful in the treatment of a
variety of tumors. (See O'Reilly et al. (1996) *Nature Med.*
2:689).

In other embodiments, gene transfer vectors can be
20 constructed to encode a cytokine or other immunomodulatory
molecule. For example, nucleic acid sequences encoding
native IL-2 and gamma-interferon can be obtained as
described in US Patent Nos. 4,738,927 and 5,326,859,
respectively, while useful muteins of these proteins can be
25 obtained as described in U.S. Patent No. 4,853,332. Nucleic
acid sequences encoding the short and long forms of mCSF can
be obtained as described in US Patent Nos. 4,847,201 and
4,879,227, respectively. In particular aspects of the
invention, retroviral vectors expressing cytokine or
30 immunomodulatory genes can be produced as described herein
(for example, employing the packaging cell lines of the

present invention) and in International Application No. PCT US 94/02951, entitled "Compositions and Methods for Cancer Immunotherapy."

5 Examples of suitable immunomodulatory molecules for use herein include the following: IL-1 and IL-2 (Karupiah et al. (1990) *J. Immunology* 144:290-298, Weber et al. (1987) *J. Exp. Med.* 166:1716-1733, Gansbacher et al. (1990) *J. Exp. Med.* 172:1217-1224, and U.S. Patent No. 4,738,927); IL-3 and IL-4 (Tepper et al. (1989) *Cell* 57:503-512, Golumbek et al. 10 (1991) *Science* 254:713-716, and U.S. Patent No. 5,017,691); IL-5 and IL-6 (Brakenhof et al. (1987) *J. Immunol.* 139:4116-4121, and International Publication No. WO 90/06370); IL-7 (U.S. Patent No. 4,965,195); IL-8, IL-9, IL-10, IL-11, IL-12, and IL-13 (*Cytokine Bulletin*, Summer 1994); IL-14 and 15 IL-15; alpha interferon (Finter et al. (1991) *Drugs* 42:749-765, U.S. Patent Nos. 4,892,743 and 4,966,843, International Publication No. WO 85/02862, Nagata et al. (1980) *Nature* 284:316-320, Familletti et al. (1981) *Methods in Enz.* 78:387-394, Twu et al. (1989) *Proc. Natl. Acad. Sci. USA* 20 86:2046-2050, and Faktor et al. (1990) *Oncogene* 5:867-872); beta-interferon (Seif et al. (1991) *J. Virol.* 65:664-671); gamma-interferons (Radford et al. (1991) *The American Society of Hepatology* 20082015, Watanabe et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:9456-9460, Gansbacher et al. (1990) 25 *Cancer Research* 50:7820-7825, Maio et al. (1989) *Can. Immunol. Immunother.* 30:34-42, and U.S. Patent Nos. 4,762,791 and 4,727,138); G-CSF (U.S. Patent Nos. 4,999,291 and 4,810,643); GM-CSF (International Publication No. WO 85/04188); tumor necrosis factors (TNFs) (Jayaraman et al. 30 (1990) *J. Immunology* 144:942-951); CD3 (Krissanen et al. (1987) *Immunogenetics* 26:258-266); ICAM-1 (Altman et al.

(1989) *Nature* 338:512-514, Simmons et al. (1988) *Nature* 331:624-627); ICAM-2, LFA-1, LFA-3 (Wallner et al. (1987) *J. Exp. Med.* 166:923-932); MHC class I molecules, MHC class II molecules, B7.1-.3, β_2 -microglobulin (Parnes et al. (1981) *Proc. Natl. Acad. Sci. USA* 78:2253-2257); chaperones such as calnexin; and MHC-linked transporter proteins or analogs thereof (Powis et al. (1991) *Nature* 354:528-531).

Immunomodulatory factors may also be agonists, antagonists, or ligands for these molecules. For example, soluble forms of receptors can often behave as antagonists for these types of factors, as can mutated forms of the factors themselves.

Nucleic acid molecules that encode the above-described substances, as well as other nucleic acid molecules that are advantageous for use within the present invention, may be readily obtained from a variety of sources, including, for example, depositories such as the American Type Culture Collection, or from commercial sources such as British Biotechnology Limited (Cowley, Oxford England). Representative examples include BBG 12 (containing the GM-CSF gene coding for the mature protein of 127 amino acids), BBG 6 (which contains sequences encoding gamma interferon), A.T.C.C. Deposit No. 39656 (which contains sequences encoding TNF), A.T.C.C. Deposit No. 20663 (which contains sequences encoding alpha-interferon), A.T.C.C. Deposit Nos. 31902, 31902 and 39517 (which contain sequences encoding beta-interferon), A.T.C.C. Deposit No. 67024 (which contains a sequence which encodes Interleukin-1b), A.T.C.C. Deposit Nos. 39405, 39452, 39516, 39626 and 39673 (which contain sequences encoding Interleukin-2), A.T.C.C. Deposit Nos. 59399, 59398, and 67326 (which contain sequences encoding Interleukin-3), A.T.C.C. Deposit No. 57592 (which contains

sequences encoding Interleukin-4), A.T.C.C. Deposit Nos. 59394 and 59395 (which contain sequences encoding Interleukin-5), and A.T.C.C. Deposit No. 67153 (which contains sequences encoding Interleukin-6).

5 Plasmids containing cytokine genes or immunomodulatory genes (International Publication Nos. WO 94/02951 and WO 96/21015, both of which are incorporated by reference in their entirety) can be digested with appropriate restriction enzymes, and DNA fragments containing the particular gene of
10 interest can be inserted into a gene transfer vector using standard molecular biology techniques. (See, e.g., Sambrook et al., *supra.*, or Ausubel et al. (eds) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley-Interscience).

15 Exemplary hormones, growth factors and other proteins which are useful for long term expression are described, for example, in European Publication No. 0437478B1, entitled "Cyclodextrin-Peptide Complexes." Nucleic acid sequences encoding a variety of hormones can be used, including those
20 encoding human growth hormone, insulin, calcitonin, prolactin, follicle stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (HCG), and thyroid stimulating hormone (TSH). A variety of different forms of IGF-1 and IGF-2 growth factor polypeptides are also
25 well known the art and can be incorporated into gene transfer vectors for long term expression *in vivo*. See, e.g., European Patent No. 0123228B1, published for grant September 19, 1993, entitled "Hybrid DNA Synthesis of Mature Insulin-like Growth Factors." As an additional example, the
30 long term *in vivo* expression of different forms of fibroblast growth factor can also be effected employing the

compositions and methods of invention. See, e.g., U.S. Patent Nos. 5,464,774, 5,155,214, and 4,994,559 for a description of different fibroblast growth factors.

Polynucleotide sequences coding for the above-described molecules can be obtained using recombinant methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene from a vector known to include the same. For example, plasmids which contain sequences that encode altered cellular products may be obtained from a depository such as the A.T.C.C., or from commercial sources. Plasmids containing the nucleotide sequences of interest can be digested with appropriate restriction enzymes, and DNA fragments containing the nucleotide sequences can be inserted into a gene transfer vector using standard molecular biology techniques.

Alternatively, cDNA sequences for use with the present invention may be obtained from cells which express or contain the sequences, using standard techniques, such as phenol extraction and PCR of cDNA or genomic DNA. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain and isolate DNA. Briefly, mRNA from a cell which expresses the gene of interest can be reverse transcribed with reverse transcriptase using oligo-dT or random primers. The single stranded cDNA may then be amplified by PCR (see U.S. Patent Nos. 4,683,202, 4,683,195 and 4,800,159, see also *PCR Technology: Principles and Applications for DNA Amplification*, Erlich (ed.), Stockton Press, 1989)) using oligonucleotide primers complementary to sequences on either side of desired sequences.

The nucleotide sequence of interest can also be produced synthetically, rather than cloned, using a DNA

synthesizer (e.g., an Applied Biosystems Model 392 DNA Synthesizer, available from ABI, Foster City, California). The nucleotide sequence can be designed with the appropriate codons for the expression product desired. The complete
5 sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge (1981) *Nature* 292:756; Nambair et al. (1984) *Science* 223:1299; Jay et al. (1984) *J. Biol. Chem.* 259:6311.

10 The synthetic expression cassettes of the present invention can be employed in the construction of packaging cell lines for use with retroviral vectors.

One type of retrovirus, the murine leukemia virus, or "MLV", has been widely utilized for gene therapy
15 applications (see generally Mann et al. (*Cell* 33:153, 1993), Cane and Mulligan (*Proc, Nat'l. Acad. Sci. USA* 81:6349, 1984), and Miller et al., *Human Gene Therapy* 1:5-14, 1990).

Lentiviral vectors typically, comprise a 5' lentiviral LTR, a tRNA binding site, a packaging signal, a promoter
20 operably linked to one or more genes of interest, an origin of second strand DNA synthesis and a 3' lentiviral LTR, wherein the lentiviral vector contains a nuclear transport element. The nuclear transport element may be located either upstream (5') or downstream (3') of a coding sequence
25 of interest. Within certain embodiments, the nuclear transport element is not RRE. Within one embodiment the packaging signal is an extended packaging signal. Within other embodiments the promoter is a tissue specific promoter, or, alternatively, a promoter such as CMV. Within
30 other embodiments, the lentiviral vector further comprises an internal ribosome entry site.

A wide variety of lentiviruses may be utilized within the context of the present invention, including for example, lentiviruses selected from the group consisting of HIV, HIV-1, HIV-2, FIV and SIV.

5 In one embodiment of the present invention synthetic Env and/or Gag-polymerase expression cassettes are provided comprising a promoter and a sequence encoding synthetic Gag-polymerase (SEQ ID NO:6) and at least one of vpr, vpu, nef or vif, wherein the promoter is operably linked to Gag-
10 polymerase and vpr, vpu, nef or vif.

Within yet another aspect of the invention, host cells (e.g., packaging cell lines) are provided which contain any of the expression cassettes described herein. For example, within one aspect packaging cell line are provided
15 comprising an expression cassette that comprises a sequence encoding synthetic Env and/or Gag-polymerase, and a nuclear transport element, wherein the promoter is operably linked to the sequence encoding Env and/or Gag-polymerase. Packaging cell lines may further comprise a promoter and a
20 sequence encoding tat, rev, or an envelope, wherein the promoter is operably linked to the sequence encoding tat, rev, or, the envelope. The packaging cell line may further comprise a sequence encoding any one or more of nef, vif, vpu or vpr.

25 In one embodiment, the expression cassette (carrying, for example, the synthetic Env, synthetic tat and/or synthetic Gag-polymerase) is stably integrated. The packaging cell line, upon introduction of a lentiviral vector, typically produces viral particles. The promoter
30 regulating expression of the synthetic expression cassette may be inducible. Typically, the packaging cell line, upon introduction of a lentiviral vector, produces viral

particles that are essentially free of replication competent virus.

Packaging cell lines are provided comprising an expression cassette which directs the expression of a synthetic *Env* (or *Gag-polymerase*) gene, an expression cassette which directs the expression of a *Gag* (or *Env*) gene optimized for expression (e.g., Andre, S., et al., *Journal of Virology* 72(2):1497-1503, 1998; Haas, J., et al., *Current Biology* 6(3):315-324, 1996). A lentiviral vector is introduced into the packaging cell line to produce a vector particle producing cell line.

As noted above, lentiviral vectors can be designed to carry or express a selected gene(s) or sequences of interest. Lentiviral vectors may be readily constructed from a wide variety of lentiviruses (see RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985). Representative examples of lentiviruses included HIV, HIV-1, HIV-2, FIV and SIV. Such lentiviruses may either be obtained from patient isolates, or, more preferably, from depositories or collections such as the American Type Culture Collection, or isolated from known sources using available techniques.

Portions of the lentiviral gene delivery vectors (or vehicles) may be derived from different viruses. For example, in a given recombinant lentiviral vector, LTRs may be derived from an HIV, a packaging signal from SIV, and an origin of second strand synthesis from HrV-2. Lentiviral vector constructs may comprise a 5' lentiviral LTR, a tRNA binding site, a packaging signal, one or more heterologous sequences, an origin of second strand DNA synthesis and a 3'

LTR, wherein said lentiviral vector contains a nuclear transport element that is not RRE.

5 Briefly, Long Terminal Repeats ("LTRs") are subdivided into three elements, designated U5, R and U3. These elements contain a variety of signals which are responsible for the biological activity of a retrovirus, including for example, promoter and enhancer elements which are located within U3. LTRs may be readily identified in the provirus (integrated DNA form) due to their precise duplication at
10 either end of the genome. As utilized herein, a 5' LTR should be understood to include a 5' promoter element and sufficient LTR sequence to allow reverse transcription and integration of the DNA form of the vector. The 3' LTR should be understood to include a polyadenylation signal,
15 and sufficient LTR sequence to allow reverse transcription and integration of the DNA form of the vector.

The tRNA binding site and origin of second strand DNA synthesis are also important for a retrovirus to be biologically active, and may be readily identified by one of
20 skill in the art. For example, retroviral tRNA binds to a tRNA binding site by Watson-Crick base pairing, and is carried with the retrovirus genome into a viral particle. The tRNA is then utilized as a primer for DNA synthesis by reverse transcriptase. The tRNA binding site may be readily
25 identified based upon its location just downstream from the 5'LTR. Similarly, the origin of second strand DNA synthesis is, as its name implies, important for the second strand DNA synthesis of a retrovirus. This region, which is also referred to as the poly-purine tract, is located just
30 upstream of the 3'LTR.

In addition to a 5' and 3' LTR, tRNA binding site, and origin of second strand DNA synthesis, recombinant

retroviral vector constructs may also comprise a packaging
signal, as well as one or more genes or coding sequences of
interest. In addition, the lentiviral vectors have a
nuclear transport element which, in preferred embodiments is
5 not RRE. Representative examples of suitable nuclear
transport elements include the element in Rous sarcoma virus
(Ogert, et al., *J ViroL* 70, 3834-3843, 1996), the element in
Rous sarcoma virus (Liu & Mertz, *Genes & Dev.*, 9, 1766-1789,
1995) and the element in the genome of simian retrovirus
10 type I (Zolotukhin, et al., *J Virol.* 68, 7944-7952, 1994).
Other potential elements include the elements in the histone
gene (Kedes, *Annu. Rev. Biochem.* 48, 837-870, 1970), the α -
interferon gene (Nagata et al., *Nature* 287, 401-408, 1980),
the β -adrenergic receptor gene (Koilkka, et al., *Nature* 329,
15 75-79, 1987), and the c-Jun gene (Hattorie, et al., *Proc.*
Natl. Acad. Sci. USA 85, 9148-9152, 1988).

Recombinant lentiviral vector constructs typically lack
both *Gag-polymerase* and *env* coding sequences. Recombinant
lentiviral vector typically contain less than 20, preferably
20 15, more preferably 10, and most preferably 8 consecutive
nucleotides found in *Gag-polymerase* or *env* genes. One
advantage of the present invention is that the synthetic
Gag-polymerase expression cassettes, which can be used to
construct packaging cell lines for the recombinant
25 retroviral vector constructs, have little homology to wild-
type *Gag-polymerase* sequences and thus considerably reduce
or eliminate the possibility of homologous recombination
between the synthetic and wild-type sequences.

Lentiviral vectors may also include tissue-specific
30 promoters to drive expression of one or more genes or
sequences of interest. For example, lentiviral vector

particles of the invention can contain a liver specific promoter to maximize the potential for liver specific expression of the exogenous DNA sequence contained in the vectors. Preferred liver specific promoters include the hepatitis B X-gene promoter and the hepatitis B core protein promoter. These liver specific promoters are preferably employed with their respective enhancers. The enhancer element can be linked at either the 5' or the 3' end of the nucleic acid encoding the sequences of interest. The hepatitis B X gene promoter and its enhancer can be obtained from the viral genome as a 332 base pair *EcoRV-NcoI* DNA fragment employing the methods described in Twu, et al., *J Virol.* 61:3448-3453, 1987. The hepatitis B core protein promoter can be obtained from the viral genome as a 584 base pair *BamHI-BglIII* DNA fragment employing the methods described in Gerlach, et al., *Virology* 189:59-66, 1992. It may be necessary to remove the negative regulatory sequence in the *BamHI-BglIII* fragment prior to inserting it. Other liver specific promoters include the AFP (alpha fetal protein) gene promoter and the albumin gene promoter, as disclosed in EP Patent Publication 0 415 731, the -1 antitrypsin gene promoter, as disclosed in Rettenger, et al., *Proc. Natl. Acad. Sci.* 91:1460-1464, 1994, the fibrinogen gene promoter, the APO-A1 (Apolipoprotein A1) gene promoter, and the promoter genes for liver transference enzymes such as, for example, SGOT, SGPT and glutamyle transferase. See also PCT Patent Publications WO 90/07936 and WO 91/02805 for a description of the use of liver specific promoters in lentiviral vector particles.

Lentiviral vector constructs may be generated such that more than one gene of interest is expressed. This may be

accomplished through the use of di- or oligo-cistronic
cassettes (e.g., where the coding regions are separated by
80 nucleotides or less, see generally Levin et al., *Gene*
108:167-174, 1991), or through the use of Internal Ribosome
5 Entry Sites ("IRES").

Packaging cell lines suitable for use with the above
described recombinant retroviral vector constructs may be
readily prepared given the disclosure provided herein.
Briefly, the parent cell line from which the packaging cell
10 line is derived can be selected from a variety of mammalian
cell lines, including for example, 293, RD, COS-7, CHO, BHK,
VERO, HT1080, and myeloma cells.

After selection of a suitable host cell for the
generation of a packaging cell line, one or more expression
15 cassettes are introduced into the cell line in order to
complement or supply in *trans* components of the vector which
have been deleted.

Representative examples of suitable expression
cassettes have been described herein and include synthetic
20 Env, tat, Gag, synthetic Gag-protease, synthetic Gag-reverse
transcriptase and synthetic Gag-polymerase expression
cassettes, which comprise a promoter and a sequence
encoding, e.g., Env, tat, or Gag-polymerase and at least one
of vpr, vpu, nef or vif, wherein the promoter is operably
25 linked to Env, tat or Gag-polymerase and vpr, vpu, nef or
vif. As described above, optimized Env, Gag and/or tat
coding sequences may also be utilized in various
combinations in the generation of packaging cell lines.

Utilizing the above-described expression cassettes, a
30 wide variety of packaging cell lines can be generated. For
example, within one aspect packaging cell line are provided

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comprising an expression cassette that comprises a sequence encoding synthetic HIV (e.g., Gag, Env, tat, Gag-polymerase, Gag-reverse transcriptase or Gag-protease) polypeptide, and a nuclear transport element, wherein the promoter is

5 operably linked to the sequence encoding the HIV polypeptide. Within other aspects, packaging cell lines are provided comprising a promoter and a sequence encoding Gag, tat, rev, or an envelope (e.g., HIV env), wherein the promoter is operably linked to the sequence encoding Gag,

10 tat, rev, or, the envelope. Within further embodiments, the packaging cell line may comprise a sequence encoding any one or more of nef, vif, vpu or vpr. For example, the packaging cell line may contain only nef, vif, vpu, or vpr alone, nef and vif, nef and vpu, nef and vpr, vif and vpu, vif and vpr,

15 vpu and vpr, nef vif and vpu, nef vif and vpr, nef vpu and vpr, vvir vpu and vpr, or, all four of nef vif vpu and vpr.

In one embodiment, the expression cassette is stably integrated. Within another embodiment, the packaging cell line, upon introduction of a lentiviral vector, produces

20 particles. Within further embodiments the promoter is inducible. Within certain preferred embodiments of the invention, the packaging cell line, upon introduction of a lentiviral vector, produces particles that are free of replication competent virus.

25 The synthetic cassettes containing optimized coding sequences are transfected into a selected cell line. Transfected cells are selected that (i) carry, typically, integrated, stable copies of the Gag, Pol, and Env coding sequences, and (ii) are expressing acceptable levels of

30 these polypeptides (expression can be evaluated by methods known in the prior art, e.g., see Examples 1-4). The

ability of the cell line to produce VLPs may also be verified (Examples 6, 7 and 15).

A sequence of interest is constructed into a suitable viral vector as discussed above. This defective virus is then transfected into the packaging cell line. The packaging cell line provides the viral functions necessary for producing virus-like particles into which the defective viral genome, containing the sequence of interest, are packaged. These VLPs are then isolated and can be used, for example, in gene delivery or gene therapy.

Further, such packaging cell lines can also be used to produce VLPs alone, which can, for example, be used as adjuvants for administration with other antigens or in vaccine compositions. Also, co-expression of a selected sequence of interest encoding a polypeptide (for example, an antigen) in the packaging cell line can also result in the entrapment and/or association of the selected polypeptide in/with the VLPs.

2.3 DNA IMMUNIZATION AND GENE DELIVERY

A variety of polypeptide antigens can be used in the practice of the present invention. Polypeptide antigens can be included in DNA immunization constructs containing, for example, any of the synthetic expression cassettes described herein fused in-frame to a coding sequence for the polypeptide antigen, where expression of the construct results in VLPs presenting the antigen of interest. Antigens can be derived from a wide variety of viruses, bacteria, fungi, plants, protozoans and other parasites. For example, the present invention will find use for stimulating an immune response against a wide variety of

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proteins from the herpesvirus family, including proteins derived from herpes simplex virus (HSV) types 1 and 2, such as HSV-1 and HSV-2 gB, gD, gH, VP16 and VP22; antigens derived from varicella zoster virus (VZV), Epstein-Barr virus (EBV) and cytomegalovirus (CMV) including CMV gB and gH; and antigens derived from other human herpesviruses such as HHV6 and HHV7. (See, e.g. Chee et al., *Cytomegaloviruses* (J.K. McDougall, ed., Springer-Verlag 1990) pp. 125-169, for a review of the protein coding content of cytomegalovirus; McGeoch et al., *J. Gen. Virol.* (1988) 69:1531-1574, for a discussion of the various HSV-1 encoded proteins; U.S. Patent No. 5,171,568 for a discussion of HSV-1 and HSV-2 gB and gD proteins and the genes encoding therefore; Baer et al., *Nature* (1984) 310:207-211, for the identification of protein coding sequences in an EBV genome; and Davison and Scott, *J. Gen. Virol.* (1986) 67:1759-1816, for a review of VZV.)

Additionally, immune responses to antigens from the hepatitis family of viruses, including hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), the delta hepatitis virus (HDV), hepatitis E virus (HEV), and hepatitis G virus, can also be stimulated using the constructs of the present invention. By way of example, the HCV genome encodes several viral proteins, including E1 (also known as E) and E2 (also known as E2/NSI), which will find use with the present invention (see, Houghton et al. *Hepatology* (1991) 14:381-388, for a discussion of HCV proteins, including E1 and E2). The δ -antigen from HDV can also be used (see, e.g., U.S. Patent No. 5,389,528, for a description of the δ -antigen).

Similarly, influenza virus is another example of a virus for which the present invention will be particularly useful. Specifically, the envelope glycoproteins HA and NA of influenza A are of particular interest for generating an immune response. Numerous HA subtypes of influenza A have been identified (Kawaoka et al., *Virology* (1990) 179:759-767; Webster et al. "Antigenic variation among type A influenza viruses," p. 127-168. In: P. Palese and D.W. Kingsbury (ed.), *Genetics of influenza viruses*. Springer-Verlag, New York).

Other antigens of particular interest to be used in the practice of the present invention include antigens and polypeptides derived therefrom from human papillomavirus (HPV), such as one or more of the various early proteins including E6 and E7; tick-borne encephalitis viruses; and HIV-1 (also known as HTLV-III, LAV, ARV, etc.), including, but not limited to, antigens such as gp120, gp41, gp160, Gag and pol from a variety of isolates including, but not limited to, HIV_{IIIB}, HIV_{SF2}, HIV-1_{SF162}, HIV-1_{SF170}, HIV_{LAV}, HIV_{LAI}, HIV_{MN}, HIV-1_{CM235}, HIV-1_{US4}, other HIV-1 strains from diverse subtypes (e.g., subtypes, A through G, and O), HIV-2 strains and diverse subtypes (e.g., HIV-2_{UC1} and HIV-2_{UC2}). See, e.g., Myers, et al., Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico; Myers, et al., *Human Retroviruses and Aids, 1990*, Los Alamos, New Mexico: Los Alamos National Laboratory.

Proteins derived from other viruses will also find use in the claimed methods, such as without limitation, proteins from members of the families Picornaviridae (e.g., polioviruses, etc.); Caliciviridae; Togaviridae (e.g., rubella virus, dengue virus, etc.); Flaviviridae;

Coronaviridae; Reoviridae; Birnaviridae; Rhabdoviridae
(e.g., rabies virus, etc.); Filoviridae; Paramyxoviridae
(e.g., mumps virus, measles virus, respiratory syncytial
virus, etc.); Orthomyxoviridae (e.g., influenza virus types
5 A, B and C, etc.); Bunyaviridae; Arenaviridae; Retroviridae,
e.g., HTLV-I; HTLV-II; HIV-1; HIV-2; simian immunodeficiency
virus (SIV) among others. See, e.g. Virology, 3rd Edition
(W.K. Joklik ed. 1988); *Fundamental Virology*, 2nd Edition
(B.N. Fields and D.M. Knipe, eds. 1991; *Virology*, 3rd
10 Edition (Fields, BN, DM Knipe, PM Howley, Editors, 1996,
Lippincott-Raven, Philadelphia, PA) for a description of
these and other viruses.

Particularly preferred bacterial antigens are derived
from organisms that cause diphtheria, tetanus, pertussis,
15 meningitis, and other pathogenic states, including, without
limitation, antigens derived from *Corynebacterium*
diphtheriae, *Clostridium tetani*, *Bordetella pertusis*,
Neisseria meningitidis, including serotypes *Meningococcus* A,
B, C, Y and WI35 (MenA, B, C, Y and WI35), *Haemophilus*
20 *influenza* type B (Hib), and *Helicobacter pylori*. Examples
of parasitic antigens include those derived from organisms
causing malaria, tuberculosis, and Lyme disease.

Furthermore, the methods described herein provide means
for treating a variety of malignant cancers. For example,
25 the system of the present invention can be used to enhance
both humoral and cell-mediated immune responses to
particular proteins specific to a cancer in question, such
as an activated oncogene, a fetal antigen, or an activation
marker. Such tumor antigens include any of the various
30 MAGEs (melanoma associated antigen E), including MAGE 1, 2,
3, 4, etc. (Boon, T. *Scientific American* (March 1993):82-

89); any of the various tyrosinases; MART 1 (melanoma antigen recognized by T cells), mutant ras; mutant p53; p97 melanoma antigen; CEA (carcinoembryonic antigen), among others.

5 DNA immunization using synthetic expression cassettes of the present invention has been demonstrated to be efficacious (Examples 8 and 10-12). Animals were immunized with both the synthetic expression cassette and the wild type expression cassette. The results of the immunizations
10 with plasmid-DNAs showed that the synthetic expression cassettes provide a clear improvement of immunogenicity relative to the native expression cassettes. Also, the second boost immunization induced a secondary immune response, for example after two to eight weeks. Further,
15 the results of CTL assays showed increased potency of synthetic expression cassettes for induction of cytotoxic T-lymphocyte (CTL) responses by DNA immunization.

It is readily apparent that the subject invention can be used to mount an immune response to a wide variety of
20 antigens and hence to treat or prevent a large number of diseases.

2.3.1 DELIVERY OF THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT INVENTION

25 Polynucleotide sequences coding for the above-described molecules can be obtained using recombinant methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene from a vector known to include the same. The sequences can be analyzed by
30 conventional sequencing techniques. Furthermore, the desired gene can be isolated directly from cells and tissues

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containing the same, using standard techniques, such as phenol extraction and PCR of cDNA or genomic DNA. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain, isolate and sequence DNA. Once the sequence is known, the gene of interest can also be produced synthetically, rather than cloned. The nucleotide sequence can be designed with the appropriate codons for the particular amino acid sequence desired. In general, one will select preferred codons for the intended host in which the sequence will be expressed. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature* (1981) 292:756; Nambair et al., *Science* (1984) 223:1299; Jay et al., *J. Biol. Chem.* (1984) 259:6311; Stemmer, W.P.C., (1995) *Gene* 164:49-53.

Next, the gene sequence encoding the desired antigen can be inserted into a vector containing a synthetic expression cassette of the present invention (e.g., see Example 1 for construction of various exemplary synthetic expression cassette). The antigen is inserted into the synthetic coding sequence such that when the combined sequence is expressed it results in the production of VLPs comprising the polypeptide and/or the antigen of interest. Insertions can be made within the Gag coding sequence or at either end of the coding sequence (5', amino terminus of the expressed polypeptide; or 3', carboxy terminus of the expressed polypeptide -- e.g., see Example 1) (Wagner, R., et al., *Arch Virol.* 127:117-137, 1992; Wagner, R., et al., *Virology* 200:162-175, 1994; Wu, X., et al., *J. Virol.* 69(6):3389-3398, 1995; Wang, C-T., et al., *Virology* 200:524-534, 1994; Chazal, N., et al., *Virology* 68(1):111-122, 1994;

Griffiths, J.C., et al., *J. Virol.* 67(6):3191-3198, 1993;
Reicin, A.S., et al., *J. Virol.* 69(2):642-650, 1995).

Up to 50% of the coding sequences of p55Gag can be
deleted without affecting the assembly to virus-like
5 particles and expression efficiency (Borsetti, A., et al,
J. Virol. 72(11):9313-9317, 1998; Gamier, L., et al., *J*
Virol 72(6):4667-4677, 1998; Zhang, Y., et al., *J Virol*
72(3):1782-1789, 1998; Wang, C., et al., *J Virol* 72(10):
7950-7959, 1998). In one embodiment of the present
10 invention, immunogenicity of the high level expressing
synthetic p55GagMod and p55GagProtMod expression cassettes
can be increased by the insertion of different structural or
non-structural HIV antigens, multiepitope cassettes, or
cytokine sequences into deleted, mutated or truncated
15 regions of p55GagMod sequence. In another embodiment of the
present invention, immunogenicity of the high level
expressing synthetic Env expression cassettes can be
increased by the insertion of different structural or non-
structural HIV antigens, multiepitope cassettes, or cytokine
20 sequences into deleted regions of gp120Mod, gp140Mod or
gp160Mod sequences. Such deletions may be generated
following the teachings of the present invention and
information available to one of ordinary skill in the art.
One possible advantage of this approach, relative to using
25 full-length modified Env sequences fused to heterologous
polypeptides, can be higher expression/secretion efficiency
and/or higher immunogenicity of the expression product. Such
deletions may be generated following the teachings of the
present invention and information available to one of
30 ordinary skill in the art. One possible advantage of this
approach, relative to using full-length Env, Gag or Tat

sequences fused to heterologous polypeptides, can be higher expression/secretion efficiency and/or immunogenicity of the expression product.

When sequences are added to the amino terminal end of
5 Gag (for example, when using the synthetic p55GagMod
expression cassette of the present invention), the
polynucleotide can contain coding sequences at the 5' end
that encode a signal for addition of a myristic moiety to
the Gag-containing polypeptide (e.g., sequences that encode
10 Met-Gly).

The ability of Gag-containing polypeptide constructs to
form VLPs can be empirically determined following the
teachings of the present specification.

HIV polypeptide/antigen synthetic expression cassettes
15 include control elements operably linked to the coding
sequence, which allow for the expression of the gene *in vivo*
in the subject species. For example, typical promoters for
mammalian cell expression include the SV40 early promoter, a
CMV promoter such as the CMV immediate early promoter, the
20 mouse mammary tumor virus LTR promoter, the adenovirus major
late promoter (Ad MLP), and the herpes simplex virus
promoter, among others. Other nonviral promoters, such as a
promoter derived from the murine metallothionein gene, will
also find use for mammalian expression. Typically,
25 transcription termination and polyadenylation sequences will
also be present, located 3' to the translation stop codon.
Preferably, a sequence for optimization of initiation of
translation, located 5' to the coding sequence, is also
present. Examples of transcription
30 terminator/polyadenylation signals include those derived

from SV40, as described in Sambrook et al., *supra*, as well as a bovine growth hormone terminator sequence.

Enhancer elements may also be used herein to increase expression levels of the mammalian constructs. Examples
5 include the SV40 early gene enhancer, as described in Dijkema et al., *EMBO J.* (1985) 4:761, the enhancer/promoter derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus, as described in Gorman et al., *Proc. Natl. Acad. Sci. USA* (1982b) 79:6777 and elements derived from
10 human CMV, as described in Boshart et al., *Cell* (1985) 41:521, such as elements included in the CMV intron A sequence.

Furthermore, plasmids can be constructed which include a chimeric antigen-coding gene sequences, encoding, e.g.,
15 multiple antigens/epitopes of interest, for example derived from a single or from more than one viral isolate.

Typically the antigen coding sequences precede or follow the synthetic coding sequences and the chimeric transcription unit will have a single open reading frame
20 encoding both the antigen of interest and the synthetic Gag coding sequences. Alternatively, multi-cistronic cassettes (e.g., bi-cistronic cassettes) can be constructed allowing expression of multiple antigens from a single mRNA using the EMCV IRES, or the like. Lastly, antigens can be encoded on
25 separate transcripts from independent promoters on a single plasmid or other vector.

Once complete, the constructs are used for nucleic acid immunization or the like using standard gene delivery protocols. Methods for gene delivery are known in the art.
30 See, e.g., U.S. Patent Nos. 5,399,346, 5,580,859, 5,589,466. Genes can be delivered either directly to the vertebrate

subject or, alternatively, delivered *ex vivo*, to cells derived from the subject and the cells reimplanted in the subject.

5 A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. Selected sequences can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and
10 delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems have been described (U.S. Patent No. 5,219,740; Miller and Rosman, *BioTechniques* (1989) 7:980-990; Miller, A.D., *Human Gene Therapy* (1990) 1:5-14; Scarpa et al., *Virology* (1991) 180:849-852; Burns et
15 al., *Proc. Natl. Acad. Sci. USA* (1993) 90:8033-8037; and Boris-Lawrie and Temin, *Cur. Opin. Genet. Develop.* (1993) 3:102-109.

A number of adenovirus vectors have also been described. Unlike retroviruses which integrate into the
20 host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and Graham, *J. Virol.* (1986) 57:267-274; Bett et al., *J. Virol.* (1993) 67:5911-5921; Mittereder et al., *Human Gene Therapy* (1994) 5:717-729; Seth et al., *J. Virol.* (1994)
25 68:933-940; Barr et al., *Gene Therapy* (1994) 1:51-58; Berkner, K.L. *BioTechniques* (1988) 6:616-629; and Rich et al., *Human Gene Therapy* (1993) 4:461-476).

Additionally, various adeno-associated virus (AAV) vector systems have been developed for gene delivery. AAV
30 vectors can be readily constructed using techniques well known in the art. See, e.g., U.S. Patent Nos. 5,173,414 and

5,139,941; International Publication Nos. WO 92/01070
(published 23 January 1992) and WO 93/03769 (published 4
March 1993); Lebkowski et al., *Molec. Cell. Biol.* (1988)
8:3988-3996; Vincent et al., *Vaccines* 90 (1990) (Cold Spring
5 Harbor Laboratory Press); Carter, B.J. *Current Opinion in*
Biotechnology (1992) 3:533-539; Muzyczka, N. *Current Topics*
in Microbiol. and Immunol. (1992) 158:97-129; Kotin, R.M.
Human Gene Therapy (1994) 5:793-801; Shelling and Smith,
Gene Therapy (1994) 1:165-169; and Zhou et al., *J. Exp. Med.*
10 (1994) 179:1867-1875.

Another vector system useful for delivering the
polynucleotides of the present invention is the enterically
administered recombinant poxvirus vaccines described by
Small, Jr., P.A., et al. (U.S. Patent No. 5,676,950, issued
15 October 14, 1997, herein incorporated by reference).

Additional viral vectors which will find use for
delivering the nucleic acid molecules encoding the antigens
of interest include those derived from the pox family of
viruses, including vaccinia virus and avian poxvirus. By
20 way of example, vaccinia virus recombinants expressing the
genes can be constructed as follows. The DNA encoding the
particular synthetic Gag/antigen coding sequence is first
inserted into an appropriate vector so that it is adjacent
to a vaccinia promoter and flanking vaccinia DNA sequences,
25 such as the sequence encoding thymidine kinase (TK). This
vector is then used to transfect cells which are
simultaneously infected with vaccinia. Homologous
recombination serves to insert the vaccinia promoter plus
the gene encoding the coding sequences of interest into the
30 viral genome. The resulting TK⁻recombinant can be selected
by culturing the cells in the presence of 5-

bromodeoxyuridine and picking viral plaques resistant thereto.

Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver the genes.

5 Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer protective immunity when administered to non-avian species. The use of an avipox vector is particularly desirable in human and other mammalian species since members of the avipox genus can only
10 productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia viruses. See, e.g.,
15 WO 91/12882; WO 89/03429; and WO 92/03545.

Molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael et al., *J. Biol. Chem.* (1993) 268:6866-6869 and Wagner et al., *Proc. Natl. Acad. Sci. USA* (1992) 89:6099-6103, can also be used for gene
20 delivery.

Members of the Alphavirus genus, such as, but not limited to, vectors derived from the Sindbis, Semliki Forest, and Venezuelan Equine Encephalitis viruses, will also find use as viral vectors for delivering the
25 polynucleotides of the present invention (for example, a synthetic Gag- or Env-polypeptide encoding expression cassette as described in Example 14 below). For a description of Sindbis-virus derived vectors useful for the practice of the instant methods, see, Dubensky et al., *J. Virol.* (1996) 70:508-519; and International Publication Nos.
30 WO 95/07995 and WO 96/17072; as well as, Dubensky, Jr.,

T.W., et al., U.S. Patent No. 5,843,723, issued December 1, 1998, and Dubensky, Jr., T.W., U.S. Patent No. 5,789,245, issued August 4, 1998, both herein incorporated by reference.

5 A vaccinia based infection/transfection system can be conveniently used to provide for inducible, transient expression of the coding sequences of interest (for example, a synthetic Gag/HCV-core expression cassette) in a host cell. In this system, cells are first infected *in vitro*
10 with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the polynucleotide of interest, driven by a
15 T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities
20 of RNA and its translation products. See, e.g., Elroy-Stein and Moss, *Proc. Natl. Acad. Sci. USA* (1990) 87:6743-6747; Fuerst et al., *Proc. Natl. Acad. Sci. USA* (1986) 83:8122-8126.

 As an alternative approach to infection with vaccinia
25 or avipox virus recombinants, or to the delivery of genes using other viral vectors, an amplification system can be used that will lead to high level expression following introduction into host cells. Specifically, a T7 RNA polymerase promoter preceding the coding region for T7 RNA
30 polymerase can be engineered. Translation of RNA derived from this template will generate T7 RNA polymerase which in

turn will transcribe more template. Concomitantly, there will be a cDNA whose expression is under the control of the T7 promoter. Thus, some of the T7 RNA polymerase generated from translation of the amplification template RNA will lead to transcription of the desired gene. Because some T7 RNA polymerase is required to initiate the amplification, T7 RNA polymerase can be introduced into cells along with the template(s) to prime the transcription reaction. The polymerase can be introduced as a protein or on a plasmid encoding the RNA polymerase. For a further discussion of T7 systems and their use for transforming cells, see, e.g., International Publication No. WO 94/26911; Studier and Moffatt, *J. Mol. Biol.* (1986) 189:113-130; Deng and Wolff, *Gene* (1994) 143:245-249; Gao et al., *Biochem. Biophys. Res. Commun.* (1994) 200:1201-1206; Gao and Huang, *Nuc. Acids Res.* (1993) 21:2867-2872; Chen et al., *Nuc. Acids Res.* (1994) 22:2114-2120; and U.S. Patent No. 5,135,855.

The synthetic expression cassette of interest can also be delivered without a viral vector. For example, the synthetic expression cassette can be packaged as DNA or RNA in liposomes prior to delivery to the subject or to cells derived therefrom. Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed DNA to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight, *Biochim. Biophys. Acta.* (1991) 1097:1-17; Straubinger et al., in *Methods of Enzymology* (1983), Vol. 101, pp. 512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations, with cationic liposomes particularly preferred. Cationic liposomes have been shown
5 to mediate intracellular delivery of plasmid DNA (Felgner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416); mRNA (Malone et al., *Proc. Natl. Acad. Sci. USA* (1989) 86:6077-6081); and purified transcription factors (Debs et al., *J. Biol. Chem.* (1990) 265:10189-10192), in functional form.

10 Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416). Other
15 commercially available lipids include (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; PCT Publication No. WO
20 90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as, from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available
25 materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP
30 starting materials in appropriate ratios. Methods for

making liposomes using these materials are well known in the art.

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The liposomes can comprise multilammellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See, e.g., Straubinger et al., in *METHODS OF IMMUNOLOGY* (1983), Vol. 101, pp. 512-527; Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* (1979) 17:77; Deamer and Bangham, *Biochim. Biophys. Acta* (1976) 443:629; Ostro et al., *Biochem. Biophys. Res. Commun.* (1977) 76:836; Fraley et al., *Proc. Natl. Acad. Sci. USA* (1979) 76:3348; Enoch and Strittmatter, *Proc. Natl. Acad. Sci. USA* (1979) 76:145; Fraley et al., *J. Biol. Chem.* (1980) 255:10431; Szoka and Papahadjopoulos, *Proc. Natl. Acad. Sci. USA* (1978) 75:145; and Schaefer-Ridder et al., *Science* (1982) 215:166.

The DNA and/or protein antigen(s) can also be delivered in cochleate lipid compositions similar to those described by Papahadjopoulos et al., *Biochem. Biophys. Acta.* (1975) 394:483-491. See, also, U.S. Patent Nos. 4,663,161 and 4,871,488.

The synthetic expression cassette of interest (e.g., any of the synthetic expression cassettes described in Example 1) may also be encapsulated, adsorbed to, or associated with, particulate carriers. Such carriers present multiple copies of a selected antigen to the immune system and promote migration, trapping and retention of antigens in local lymph nodes. The particles can be taken up by profession antigen presenting cells such as

macrophages and dendritic cells, and/or can enhance antigen presentation through other mechanisms such as stimulation of cytokine release. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well
5 as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; McGee JP, et al., *J Microencapsul.* 14(2):197-210, 1997; O'Hagan DT, et al., *Vaccine* 11(2):149-54, 1993.

10 Furthermore, other particulate systems and polymers can be used for the *in vivo* or *ex vivo* delivery of the gene of interest. For example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules, are useful for
15 transferring a nucleic acid of interest. Similarly, DEAE dextran-mediated transfection, calcium phosphate precipitation or precipitation using other insoluble inorganic salts, such as strontium phosphate, aluminum silicates including bentonite and kaolin, chromic oxide,
20 magnesium silicate, talc, and the like, will find use with the present methods. See, e.g., Felgner, P.L., *Advanced Drug Delivery Reviews* (1990) 5:163-187, for a review of delivery systems useful for gene transfer. Peptoids (Zuckerman, R.N., et al., U.S. Patent No. 5,831,005, issued
25 November 3, 1998, herein incorporated by reference) may also be used for delivery of a construct of the present invention.

Additionally, biolistic delivery systems employing particulate carriers such as gold and tungsten, are
30 especially useful for delivering synthetic expression cassettes of the present invention. The particles are

coated with the synthetic expression cassette(s) to be delivered and accelerated to high velocity, generally under a reduced atmosphere, using a gun powder discharge from a "gene gun." For a description of such techniques, and apparatuses useful therefore, see, e.g., U.S. Patent Nos. 4,945,050; 5,036,006; 5,100,792; 5,179,022; 5,371,015; and 5,478,744. Also, needle-less injection systems can be used (Davis, H.L., et al, *Vaccine* 12:1503-1509, 1994; Bioject, Inc., Portland, OR).

Recombinant vectors carrying a synthetic expression cassette of the present invention are formulated into compositions for delivery to the vertebrate subject. These compositions may either be prophylactic (to prevent infection) or therapeutic (to treat disease after infection). The compositions will comprise a "therapeutically effective amount" of the gene of interest such that an amount of the antigen can be produced *in vivo* so that an immune response is generated in the individual to which it is administered. The exact amount necessary will vary depending on the subject being treated; the age and general condition of the subject to be treated; the capacity of the subject's immune system to synthesize antibodies; the degree of protection desired; the severity of the condition being treated; the particular antigen selected and its mode of administration, among other factors. An appropriate effective amount can be readily determined by one of skill in the art. Thus, a "therapeutically effective amount" will fall in a relatively broad range that can be determined through routine trials.

The compositions will generally include one or more "pharmaceutically acceptable excipients or vehicles" such as

water, saline, glycerol, polyethyleneglycol, hyaluronic acid, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, surfactants and the like, may be present in such
5 vehicles. Certain facilitators of immunogenicity or of nucleic acid uptake and/or expression can also be included in the compositions or coadministered, such as, but not limited to, bupivacaine, cardiotoxin and sucrose.

Once formulated, the compositions of the invention can
10 be administered directly to the subject (e.g., as described above) or, alternatively, delivered *ex vivo*, to cells derived from the subject, using methods such as those described above. For example, methods for the *ex vivo* delivery and reimplantation of transformed cells into a
15 subject are known in the art and can include, e.g., dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, lipofectamine and LT-1 mediated transfection, protoplast fusion, electroporation, encapsulation of the
20 polynucleotide(s) (with or without the corresponding antigen) in liposomes, and direct microinjection of the DNA into nuclei.

Direct delivery of synthetic expression cassette compositions *in vivo* will generally be accomplished with or
25 without viral vectors, as described above, by injection using either a conventional syringe, needless devices such as Bioject® or a gene gun, such as the Accell® gene delivery system (PowderJect Technologies, Inc., Oxford, England). The constructs can be delivered (e.g., injected) either
30 subcutaneously, epidermally, intradermally, intramuscularly, intravenous, intramucosally (such as nasally, rectally and

vaginally), intraperitoneally or orally. Delivery of DNA into cells of the epidermis is particularly preferred as this mode of administration provides access to skin-associated lymphoid cells and provides for a transient
5 presence of DNA in the recipient. Other modes of administration include oral ingestion and pulmonary administration, suppositories, needle-less injection, transcutaneous and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose
10 schedule.

2.3.2 EX VIVO DELIVERY OF THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT INVENTION

In one embodiment, T cells, and related cell types
15 (including but not limited to antigen presenting cells, such as, macrophage, monocytes, lymphoid cells, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof), can be used for ex vivo delivery of the synthetic expression cassettes of the present invention. T cells can be isolated
20 from peripheral blood lymphocytes (PBLs) by a variety of procedures known to those skilled in the art. For example, T cell populations can be "enriched" from a population of PBLs through the removal of accessory and B cells. In particular, T cell enrichment can be accomplished by the
25 elimination of non-T cells using anti-MHC class II monoclonal antibodies. Similarly, other antibodies can be used to deplete specific populations of non-T cells. For example, anti-Ig antibody molecules can be used to deplete B cells and anti-MacI antibody molecules can be used to
30 deplete macrophages.

5 T cells can be further fractionated into a number of
different subpopulations by techniques known to those
skilled in the art. Two major subpopulations can be
isolated based on their differential expression of the cell
surface markers CD4 and CD8. For example, following the
enrichment of T cells as described above, CD4⁺ cells can be
enriched using antibodies specific for CD4 (see Coligan et
al., *supra*). The antibodies may be coupled to a solid
support such as magnetic beads. Conversely, CD8⁺ cells can
10 be enriched through the use of antibodies specific for CD4
(to remove CD4⁺ cells), or can be isolated by the use of CD8
antibodies coupled to a solid support. CD4 lymphocytes from
HIV-1 infected patients can be expanded *ex vivo*, before or
after transduction as described by Wilson et. al. (1995) *J.*
15 *Infect. Dis.* 172:88.

Following purification of T cells, a variety of methods
of genetic modification known to those skilled in the art
can be performed using non-viral or viral-based gene
transfer vectors constructed as described herein. For
20 example, one such approach involves transduction of the
purified T cell population with vector-containing
supernatant of cultures derived from vector producing cells.
A second approach involves co-cultivation of an irradiated
monolayer of vector-producing cells with the purified T
25 cells. A third approach involves a similar co-cultivation
approach; however, the purified T cells are pre-stimulated
with various cytokines and cultured 48 hours prior to the
co-cultivation with the irradiated vector producing cells.
Pre-stimulation prior to such transduction increases
30 effective gene transfer (Nolta et al. (1992) *Exp. Hematol.*
20:1065). Stimulation of these cultures to proliferate also

provides increased cell populations for re-infusion into the patient. Subsequent to co-cultivation, T cells are collected from the vector producing cell monolayer, expanded, and frozen in liquid nitrogen.

5 Gene transfer vectors, containing one or more synthetic expression cassette of the present invention (associated with appropriate control elements for delivery to the isolated T cells) can be assembled using known methods.

10 Selectable markers can also be used in the construction of gene transfer vectors. For example, a marker can be used which imparts to a mammalian cell transduced with the gene transfer vector resistance to a cytotoxic agent. The cytotoxic agent can be, but is not limited to, neomycin, aminoglycoside, tetracycline, chloramphenicol, sulfonamide,
15 actinomycin, netropsin, distamycin A, anthracycline, or pyrazinamide. For example, neomycin phosphotransferase II imparts resistance to the neomycin analogue geneticin (G418).

20 The T cells can also be maintained in a medium containing at least one type of growth factor prior to being selected. A variety of growth factors are known in the art which sustain the growth of a particular cell type. Examples of such growth factors are cytokine mitogens such as rIL-2, IL-10, IL-12, and IL-15, which promote growth and
25 activation of lymphocytes. Certain types of cells are stimulated by other growth factors such as hormones, including human chorionic gonadotropin (hCG) and human growth hormone. The selection of an appropriate growth factor for a particular cell population is readily
30 accomplished by one of skill in the art.

For example, white blood cells such as differentiated progenitor and stem cells are stimulated by a variety of

growth factors. More particularly, IL-3, IL-4, IL-5, IL-6, IL-9, GM-CSF, M-CSF, and G-CSF, produced by activated T_H and activated macrophages, stimulate myeloid stem cells, which then differentiate into pluripotent stem cells, granulocyte-
5 monocyte progenitors, eosinophil progenitors, basophil progenitors, megakaryocytes, and erythroid progenitors. Differentiation is modulated by growth factors such as GM-CSF, IL-3, IL-6, IL-11, and EPO.

Pluripotent stem cells then differentiate into lymphoid
10 stem cells, bone marrow stromal cells, T cell progenitors, B cell progenitors, thymocytes, T_H Cells, T_C cells, and B cells. This differentiation is modulated by growth factors such as IL-3, IL-4, IL-6, IL-7, GM-CSF, M-CSF, G-CSF, IL-2, and IL-5.

15 Granulocyte-monocyte progenitors differentiate to monocytes, macrophages, and neutrophils. Such differentiation is modulated by the growth factors GM-CSF, M-CSF, and IL-8. Eosinophil progenitors differentiate into eosinophils. This process is modulated by GM-CSF and IL-5.

20 The differentiation of basophil progenitors into mast cells and basophils is modulated by GM-CSF, IL-4, and IL-9. Megakaryocytes produce platelets in response to GM-CSF, EPO, and IL-6. Erythroid progenitor cells differentiate into red blood cells in response to EPO.

25 Thus, during activation by the CD3-binding agent, T cells can also be contacted with a mitogen, for example a cytokine such as IL-2. In particularly preferred embodiments, the IL-2 is added to the population of T cells at a concentration of about 50 to 100 µg/ml. Activation
30 with the CD3-binding agent can be carried out for 2 to 4 days.

Once suitably activated, the T cells are genetically modified by contacting the same with a suitable gene transfer vector under conditions that allow for transfection of the vectors into the T cells. Genetic modification is carried out when the cell density of the T cell population is between about 0.1×10^6 and 5×10^6 , preferably between about 0.5×10^6 and 2×10^6 . A number of suitable viral and nonviral-based gene transfer vectors have been described for use herein.

After transduction, transduced cells are selected away from non-transduced cells using known techniques. For example, if the gene transfer vector used in the transduction includes a selectable marker which confers resistance to a cytotoxic agent, the cells can be contacted with the appropriate cytotoxic agent, whereby non-transduced cells can be negatively selected away from the transduced cells. If the selectable marker is a cell surface marker, the cells can be contacted with a binding agent specific for the particular cell surface marker, whereby the transduced cells can be positively selected away from the population. The selection step can also entail fluorescence-activated cell sorting (FACS) techniques, such as where FACS is used to select cells from the population containing a particular surface marker, or the selection step can entail the use of magnetically responsive particles as retrievable supports for target cell capture and/or background removal.

More particularly, positive selection of the transduced cells can be performed using a FACS cell sorter (e.g. a FACSVantage™ Cell Sorter, Becton Dickinson Immunocytometry Systems, San Jose, CA) to sort and collect transduced cells expressing a selectable cell surface marker. Following transduction, the cells are stained with fluorescent-labeled

antibody molecules directed against the particular cell surface marker. The amount of bound antibody on each cell can be measured by passing droplets containing the cells through the cell sorter. By imparting an electromagnetic charge to droplets containing the stained cells, the transduced cells can be separated from other cells. The positively selected cells are then harvested in sterile collection vessels. These cell sorting procedures are described in detail, for example, in the FACS Vantage™ Training Manual, with particular reference to sections 3-11 to 3-28 and 10-1 to 10-17.

Positive selection of the transduced cells can also be performed using magnetic separation of cells based on expression of a particular cell surface marker. In such separation techniques, cells to be positively selected are first contacted with specific binding agent (e.g., an antibody or reagent that interacts specifically with the cell surface marker). The cells are then contacted with retrievable particles (e.g., magnetically responsive particles) which are coupled with a reagent that binds the specific binding agent (that has bound to the positive cells). The cell-binding agent-particle complex can then be physically separated from non-labeled cells, for example using a magnetic field. When using magnetically responsive particles, the labeled cells can be retained in a container using a magnetic field while the negative cells are removed. These and similar separation procedures are known to those of ordinary skill in the art.

Expression of the vector in the selected transduced cells can be assessed by a number of assays known to those skilled in the art. For example, Western blot or Northern analysis can be employed depending on the nature of the

inserted nucleotide sequence of interest. Once expression has been established and the transformed T cells have been tested for the presence of the selected synthetic expression cassette, they are ready for infusion into a patient via the peripheral blood stream.

The invention includes a kit for genetic modification of an ex vivo population of primary mammalian cells. The kit typically contains a gene transfer vector coding for at least one selectable marker and at least one synthetic expression cassette contained in one or more containers, ancillary reagents or hardware, and instructions for use of the kit.

EXPERIMENTAL

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

Example 1

Generation of Synthetic Gag and Env Expression Cassettes

A. Modification of HIV-1 Gag, Gag-protease, Gag-reverse transcriptase and Gag-polymerase Nucleic Acid Coding Sequences

The Gag (SEQ ID NO:1), Gag-protease (SEQ ID NO:2), Gag-polymerase (SEQ ID NO:3), and Gag-reverse transcriptase (SEQ ID NO:77) coding sequences were selected from the HIV-1SF2 strain (Sanchez-Pescador, R., et al., *Science* 227(4686): 484-492, 1985; Luciw, P.A., et al. U.S. Patent No. 5,156,949, issued October 20, 1992, herein incorporated by reference; Luciw, P.A., et al., U.S. Patent No. 5,688,688, November 18, 1997). These sequences were manipulated to maximize expression of their gene products.

First, the HIV-1 codon usage pattern was modified so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes. The HIV codon usage reflects a high content of the nucleotides A or T of the codon-triplet. The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a high AU content in the RNA and in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Gag-encoding sequences were modified to be comparable to codon usage found in highly expressed human genes.

Figure 11 presents a comparison of the percent A-T content for the cDNAs of stable versus unstable RNAs (comparison window size = 50). Human IFN γ mRNA is known to (i) be unstable, (ii) have a short half-life, and (iii) have

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a high A-U content. Human GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA is known to (i) be a stable RNA, and (i) have a low A-U content. In Figure 11, the percent A-T content of these two sequences are compared to the percent A-T content of native HIV-1SF2 Gag cDNA and to the synthetic Gag cDNA sequence of the present invention. The top two panels of the figure show the percent A-T content over the length of the sequences for IFN γ and native Gag. The bottom two panels of the figure show the percent A-T content over the length of the sequences for GAPDH and the synthetic Gag. Experiments performed in support of the present invention showed that the synthetic Gag sequences were capable of higher level of protein production (see the Examples) than the native Gag sequences. The data in Figure 11 suggest that one reason for this increased production may be increased stability of the mRNA corresponding to the synthetic Gag coding sequences versus the mRNA corresponding to the native Gag coding sequences.

Second, there are inhibitory (or instability) elements (INS) located within the coding sequences of the Gag and Gag-protease coding sequences (Schneider R, et al., *J Virol.* 71(7):4892-4903, 1997). RRE is a secondary RNA structure that interacts with the HIV encoded Rev-protein to overcome the expression down-regulating effects of the INS. To overcome the requirement for post-transcriptional activating mechanisms of RRE and Rev, and to enhance independent expression of the Gag polypeptide, the INS were inactivated by introducing multiple point mutations that did not alter the reading frame of the encoded proteins. Figure 1 shows the original SF2 Gag sequence, the location of the INS

sequences, and the modifications made to the INS sequences to reduce their effects.

For the Gag-protease sequence (wild type, SEQ ID NO:2; synthetic, SEQ ID NOs:5, 78 and 79), the changes in codon
5 usage were restricted to the regions up to the -1 frameshift and starting again at the end of the Gag reading frame (Figure 2; the region indicated in lower case letters in Figure 2 is the unmodified region). Further, inhibitory (or instability) elements (INS) located within the coding
10 sequences of the Gag-protease polypeptide coding sequence were altered as well (indicated in Figure 2). The synthetic coding sequences were assembled by the Midland Certified Reagent Company (Midland, Texas).

Modification of the Gag-polymerase sequences (wild
15 type, SEQ ID NO:3; synthetic, SEQ ID NO:6) and Gag-reverse transcriptase sequences (SEQ ID NOs:80 through 84) include similar modifications as described for Gag-protease in order to preserve the frameshift region. Locations of the inactivation sites and changes to the sequence to alter the
20 inactivation sites are presented in Figure 12 for the native HIV-1_{SF2} Gag-polymerase sequence.

In one embodiment of the invention, the full length polymerase coding region of the Gag-polymerase sequence is included with the synthetic Gag sequences in order to
25 increase the number of epitopes for virus-like particles expressed by the synthetic, optimized Gag expression cassette. Because synthetic HIV-1 Gag-polymerase expresses the potentially deleterious functional enzymes reverse transcriptase (RT) and integrase (INT) (in addition to the
30 structural proteins and protease), it is important to inactivate RT and INT functions. Several in-frame deletions in the RT and INT reading frame can be made to achieve

- catalytic nonfunctional enzymes with respect to their RT and INT activity. {Jay. A. Levy (Editor) (1995) *The Retroviridae*, Plenum Press, New York. ISBN 0-306-45033X. Pages 215-20; Grimison, B. and Laurence, J. (1995), *Journal Of Acquired Immune Deficiency Syndromes and Human Retrovirology* 9(1):58-68; Wakefield, J. K., et al., (1992) *Journal Of Virology* 66(11):6806-6812; Esnouf, R., et al., (1995) *Nature Structural Biology* 2(4):303-308; Maignan, S., et al., (1998) *Journal Of Molecular Biology* 282(2):359-368;
- 10 Katz, R. A. and Skalka, A. M. (1994) *Annual Review Of Biochemistry* 73 (1994); Jacobo-Molina, A., et al., (1993) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 90(13):6320-6324; Hickman, A. B., et al., (1994) *Journal Of Biological Chemistry*
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the United States Of America 92(4):1222-1226; Sheng, N. and Dennis, D. (1993) *Biochemistry* 32(18):4938-4942; Spence, R. A., et al., (1995) *Science* 267(5200):988-993.}

Furthermore selected B- and/or T-cell epitopes can be
5 added to the Gag-polymerase constructs within the deletions
of the RT- and INT-coding sequence to replace and augment
any epitopes deleted by the functional modifications of RT
and INT. Alternately, selected B- and T-cell epitopes
(including CTL epitopes) from RT and INT can be included in
10 a minimal VLP formed by expression of the synthetic Gag or
synthetic GagProt cassette, described above. (For
descriptions of known HIV B- and T-cell epitopes see, HIV
Molecular Immunology Database CTL Search Interface; Los
Alamos Sequence Compendia, 1987-1997; Internet address:
15 <http://hiv-web.lanl.gov/immunology/index.html>.)

The resulting modified coding sequences are presented
as a synthetic Gag expression cassette (SEQ ID NO:4), a
synthetic Gag-protease expression cassette (SEQ ID NOs:5, 78
and 79), and a synthetic Gag-polymerase expression cassette
20 (SEQ ID NO:6). Synthetic expression cassettes containing
codon modifications in the reverse transcriptase region are
shown in SEQ ID NOs:80 through 84. An alignment of selected
sequences is presented in Figure 7. A common region (Gag-
common; SEQ ID NO:9) extends from position 1 to position
25 1262.

The synthetic DNA fragments for Gag and Gag-protease
were cloned into the following expression vectors: pCMVKm2,
for transient expression assays and DNA immunization
studies, the pCMVKm2 vector was derived from pCMV6a (Chapman
30 et al., *Nuc. Acids Res.* (1991) 19:3979-3986) and comprises a
kanamycin selectable marker, a ColE1 origin of replication,

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a CMV promoter enhancer and Intron A, followed by an insertion site for the synthetic sequences described below followed by a polyadenylation signal derived from bovine growth hormone -- the pCMVKm2 vector differs from the pCMV-link vector only in that a polylinker site was inserted into
5 pCMVKm2 to generate pCMV-link (Figure 14, polylinker at positions 1646 to 1697); pESN2dhfr (Figure 13A) and pCMVPLEdhfr (also known as pCMVIII as shown in Figure 13B), for expression in Chinese Hamster Ovary (CHO) cells; and,
10 pAcC13, a shuttle vector for use in the Baculovirus expression system (pAcC13, was derived from pAcC12 which was described by Munemitsu S., et al., *Mol Cell Biol.* 10(11):5977-5982, 1990).

A restriction map for vector pCMV-link is presented in
15 Figure 14. In the figure, the CMV promoter (CMV IE ENH/PRO), bovine growth hormone terminator (BGH pA), kanamycin selectable marker (kan), and a ColE1 origin of replication (ColE1 ori) are indicated. A polycloning site is also indicated in the figure following the CMV promoter
20 sequences.

A restriction map for vector pESN2dhfr is presented in Figure 13A. In the figure, the CMV promoter (pCMV, hCMVIE), bovine growth hormone terminator (BGHpA), SV40 origin of replication (SV40ori), neomycin selectable marker (Neo),
25 SV40 polyA (SV40pA), Adenovirus 2 late promoter (Ad2VLP), and the murine dhfr gene (mu dhfr) are indicated. A polycloning site is also indicated in the figure following the CMV promoter sequences.

Briefly, construction of pCMVPLEdhfr (pCMVIII) was as
30 follows. To construct a DHFR cassette, the EMCV IRES (internal ribosome entry site) leader was PCR-amplified from

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pCite-4a+ (Novagen, Inc., Milwaukee, WI) and inserted into
pET-23d (Novagen, Inc., Milwaukee, WI) as an *Xba*-*Nco*
fragment to give pET-EMCV. The *dhfr* gene was PCR-amplified
5 spacer in place of the translation stop codon and inserted
as an *Nco*-*Bam*H1 fragment to give pET-E-DHFR. Next, the
attenuated *neo* gene was PCR amplified from a pSV2Neo
(Clontech, Palo Alto, CA) derivative and inserted into the
unique *Bam*H1 site of pET-E-DHFR to give pET-E-DHFR/*Neo*_(m2).
10 Then, the bovine growth hormone terminator from pCDNA3
(Invitrogen, Inc., Carlsbad, CA) was inserted downstream of
the *neo* gene to give pET-E-DHFR/*Neo*_(m2)BGht. The EMCV-
dhfr/neo selectable marker cassette fragment was prepared by
cleavage of pET-E-DHFR/*Neo*_(m2)BGht. The CMV enhancer/promoter
15 plus Intron A was transferred from pCMV6a (Chapman et al.,
Nuc. Acids Res. (1991) 19:3979-3986) as a *Hind*III-*Sal*I
fragment into pUC19 (New England Biolabs, Inc., Beverly,
MA). The vector backbone of pUC19 was deleted from the *Nde*I
to the *Sap*I sites. The above described DHFR cassette was
20 added to the construct such that the EMCV IRES followed the
CMV promoter to produce the final construct. The vector
also contained an *amp*^r gene and an SV40 origin of
replication.

Selected pCMVKm2 vectors containing the synthetic
25 expression cassettes have been designated as follows:
pCMVKm2.GagMod.SF2, pCMVKm2.GagprotMod.SF2, and
pCMVKm2.GagpolMod.SF2, pCMVKm2.GagprotMod.SF2.GP1 (SEQ ID
NO:78) and pCMVKm2.GagprotMod.SF2.GP2 (SEQ ID NO:79). Other
exemplary Gag-encoding expressing cassettes are shown in the
30 Figures and as Sequence Listings.

B. Modification of HIV-1 Gag/Hepatitis C Core Chimeric Protein Nucleic Acid Coding Sequences Generation of Synthetic Expression Cassettes

To facilitate the ligation of the Gag and HCV core coding sequences, PCR amplification was employed. The synthetic p55Gag expression cassette was used as a PCR template with the following primers: GAG5 (SEQ ID NO:11) and P55-SAL3 (SEQ ID NO:12). The PCR amplification was conducted at 55°C for 25 cycles using Stratagene's Pfu polymerase. The resulting PCR product was rendered free of nucleotides and primers using the Promega PCR clean-up kit and then subjected to EcoRI and SalI digestions. For HCV core coding sequences, the following primers were used with an HCV template (Houghton, M., et al., U.S. Patent No. 5,714,596, issued February 3, 1998; Houghton, M., et al., U.S. Patent No. 5,712,088, issued January 27, 1998; Houghton, M., et al., U.S. Patent No. 5,683,864, issued November 4, 1997; Weiner, A.J., et al., U.S. Patent No. 5,728,520, issued March 17, 1998; Weiner, A.J., et al., U.S. Patent No. 5,766,845, issued June 16, 1998; Weiner, A.J., et al., U.S. Patent No. 5,670,152, issued September 23, 1997; all herein incorporated by reference): CORESAL 5 (SEQ ID NO:13) and 173CORE (SEQ ID NO:14) using the conditions outlined above. The purified product was digested with SalI and BamHI restriction enzymes. The digested Gag and HCV core PCR products were ligated into the pCMVKm2 vector digested with EcoRI and BamHI. Ligation of the PCR products at the SalI site resulted in a direct fusion of the final amino acid of p55Gag to the second amino acid of HCV core, serine. Amino acid 173 of core is a serine and is followed immediately by a TAG termination codon. The sequence of the fusion clone

was confirmed. The pCMVKm2 vector containing the synthetic expression cassette was designated as pCMVKm2.GagModHCVcore.

The EcoRI-BamHI fragment of p55Gag-core 173 was also cloned into EcoRI-BamHI-digested pAcC13 for baculovirus expression. Western blots confirmed expression and sucrose gradient sedimentation along with electron microscopy confirmed particle formation. To generate the above clone but containing the synthetic Gag sequences (instead of wild-type), the following steps were performed: pCMVKm2-modified p55Gag was used as template for PCR amplification with MS65 (SEQ ID NO:15) and MS66 (SEQ ID NO:16) primers. The region amplified corresponds to the BspHI and SalI sites at the C-terminus of synthetic Gag sequence. The amplification product was digested with BspHI and SalI and ligated to SalI/BamHI digested pCMV-link along with the Sal/BspHI fragment from pCMV-Km-p55modGag, representing the amino terminal end of modified Gag, and the SalI/BamHI fragment from pCMV-p55Gag-core173. Thereafter, a T4-blunted-SalI partial/BamHI fragment was ligated into pAcC4-SmaI/BamHI to generate pAcC4-p55GagMod-core173 (containing the synthetic sequence presented as SEQ ID NO:7).

C. Defining of the Major Homology Region (MHR) of HIV-1 p55Gag

The Major Homology Region (MHR) of HIV-1 p55 (Gag) is located in the p24-CA sequence of Gag. It is a conserved stretch of 20 amino acids (SEQ ID NO:19). The position in the wild type HIV-1_{SF2} Gag protein is from aa 286-305 and spans a region from nucleotides 856-915 in the native HIV-1_{SF2} Gag DNA-sequence. The position in the synthetic Gag protein is from aa 288-307 and spans a region from nucleotides 862-921 for the synthetic Gag DNA-sequence. The

nucleotide sequence for the MHR in the synthetic GagMod.SF2 is presented as SEQ ID NO:20. Mutations or deletions in the amino acid sequence of the MHR can severely impair particle production (Borsetti, A., et al., *J. Virol.* 72(11):9313-9317, 1998; Mammano, F., et al., *J Virol* 68(8):4927-4936, 1994).

Percent identity to the MHR nucleotide sequence can be determined, for example, using the MacDNAsis program (Hitachi Software Engineering America Limited, South San Francisco, CA), Higgins algorithm, with the following exemplary parameters: gap penalty = 5, no. of top diagonals = 5, fixed gap penalty = 5, K-tuple = 2, window size = 5, and floating gap penalty = 10.

D. Generation of Synthetic Env Expression Cassettes

Env coding sequences of the present invention include, but are not limited to, polynucleotide sequences encoding the following HIV-encoded polypeptides: gp160, gp140, and gp120 (see, e.g., U.S. Patent No. 5,792,459 for a description of the HIV-1_{SF2} ("SF2") Env polypeptide). The relationships between these polypeptides is shown schematically in Figure 15 (in the figure: the polypeptides are indicated as lines, the amino and carboxy termini are indicated on the gp160 line; the open circle represents the oligomerization domain; the open square represents a transmembrane spanning domain (TM); and "c" represents the location of a cleavage site, in gp140.mut the "X" indicates that the cleavage site has been mutated such that it no longer functions as a cleavage site). The polypeptide gp160 includes the coding sequences for gp120 and gp41. The polypeptide gp41 is comprised of several domains including

an oligomerization domain (OD) and a transmembrane spanning domain (TM). In the native envelope, the oligomerization domain is required for the non-covalent association of three gp41 polypeptides to form a trimeric structure: through
5 non-covalent interactions with the gp41 trimer (and itself), the gp120 polypeptides are also organized in a trimeric structure. A cleavage site (or cleavage sites) exists approximately between the polypeptide sequences for gp120 and the polypeptide sequences corresponding to gp41. This
10 cleavage site(s) can be mutated to prevent cleavage at the site. The resulting gp140 polypeptide corresponds to a truncated form of gp160 where the transmembrane spanning domain of gp41 has been deleted. This gp140 polypeptide can exist in both monomeric and oligomeric (*i.e.* trimeric) forms
15 by virtue of the presence of the oligomerization domain in the gp41 moiety. In the situation where the cleavage site has been mutated to prevent cleavage and the transmembrane portion of gp41 has been deleted the resulting polypeptide product is designated "mutated" gp140 (e.g., gp140.mut). As
20 will be apparent to those in the field, the cleavage site can be mutated in a variety of ways. The native amino acid sequence in the SF162 cleavage sites is: APTKAKRRVVQREKR (SEQ ID NO:21), where KAKRR (SEQ ID NO:22) is termed the "second" site and REKR (SEQ ID NO:23) is the "first site".
25 Exemplary mutations include the following constructs: gp140.mut7.modSF162 which encodes the amino acid sequence APTKAISSVVQSEKS (SEQ ID NO:24) in the cleavage site region; gp140.mut8.modSF162 which encodes the amino acid sequence APTIAISSVVQSEKS (SEQ ID NO:25) in the cleavage site region
30 and gp140mut.modSF162 which encodes the amino acid sequence APTKAKRRVVQREKS (SEQ ID NO:26). Mutations are denoted in

bold. The native amino acid sequence in the US4 cleavage sites is: APTQAKRRVVQREKR (SEQ ID NO:27), where QAKRR (SEQ ID NO:28) is termed the "second" site and REKR (SEQ ID NO:23) is the "first site". Exemplary mutations include the following construct: gp140.mut.modUS4 which encodes the amino acid sequence APTQAKRRVVQREKS (SEQ ID NO:29) in the cleavage site region. Mutations are denoted in bold.

E. Modification of HIV-1 Env (Envelope) Nucleic Acid Coding Sequences

In one embodiment of the present invention, wild-type *Env* coding sequences were selected from the HIV-1_{SF162} ("SF162") strain (Cheng-Mayer (1989) *PNAS USA* 86:8575-8579). These SF162 sequences were as follows: gp120, SEQ ID NO:30 (Fig. 16); gp140, SEQ ID NO:31 (Fig. 17); and gp160, SEQ ID NO:32 (Fig. 18).

In another embodiment of the present invention, wild-type *Env* coding sequences were selected from the HIV-US4 strain (Mascola, et al. (1994) *J. Infect. Dis.* 169:48-54).

These US4 sequences were as follows: gp120, SEQ ID NO:51 (Fig. 38); gp140, SEQ ID NO:52 (Fig. 39); and gp160, SEQ ID NO:53 (Fig. 40).

These *Env* coding sequences were manipulated to maximize expression of their gene products.

First, the wild-type coding region was modified in one or more of the following ways. In one embodiment, sequences encoding hypervariable regions of *Env*, particularly V1 and/or V2 were deleted. In other embodiments, mutations were introduced into sequences encoding the cleavage site in *Env* to abrogate the enzymatic cleavage of oligomeric gp140 into gp120 monomers. (See, e.g., Earl et al. (1990) *PNAS*

USA 87:648-652; Earl et al. (1991) *J. Virol.* 65:31-41). In yet other embodiments, hypervariable region(s) were deleted, N-glycosylation sites were removed and/or cleavage sites mutated.

5 Second, the HIV-1 codon usage pattern was modified so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes. The HIV codon usage reflects a high content of the nucleotides A or T in the codon-triplet. The effect of the
10 HIV-1 codon usage is a high AT content in the DNA sequence that results in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Env coding sequences were modified to be comparable to codon usage
15 found in highly expressed human genes.

 Figures 22A-22H present comparisons of the percent A-T content for the cDNAs of stable versus unstable RNAs (comparison window size = 50). Human IFN γ mRNA is known to
20 (i) be unstable, (ii) have a short half-life, and (iii) have a high A-U content. Human GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA is known to (i) be a stable RNA, and (i) have a low A-U content. In Figures 22A-H, the percent A-T content of these two sequences are compared to the percent A-T content of (1) native HIV-1 US4 Env gp160 cDNA, a
25 synthetic US4 Env gp160 cDNA sequence (i.e., having modified codons) of the present invention; and (2) native HIV-1 SF162 Env gp160 cDNA, a synthetic SF162 Env gp160 cDNA sequence (i.e., having modified codons) of the present invention. Figures 22A-H show the percent A-T content over the length
30 of the sequences for IFN γ (Figures 22C and 22G); native gp160 Env US4 and SF162 (Figures 22A and 22E, respectively);

GAPDH (Figures 22D and 22H); and the synthetic gp160 Env for US4 and SF162 (Figures 22B and 22F). Experiments performed in support of the present invention showed that the synthetic Env sequences were capable of higher level of protein production (see the Examples) than the native Env sequences. The data in Figures 22A-H suggest that one reason for this increased production is increased stability of the mRNA corresponding to the synthetic Env coding sequences versus the mRNA corresponding to the native Env coding sequences.

To create the synthetic coding sequences of the present invention the gene cassettes were designed to comprise the entire coding sequence of interest. Synthetic gene cassettes were constructed by oligonucleotide synthesis and PCR amplification to generate gene fragments. Primers were chosen to provide convenient restriction sites for subcloning. The resulting fragments were then ligated to create the entire desired sequence which was then cloned into an appropriate vector. The final synthetic sequences were (i) screened by restriction endonuclease digestion and analysis, (ii) subjected to DNA sequencing in order to confirm that the desired sequence had been obtained and (iii) the identity and integrity of the expressed protein confirmed by SDS-PAGE and Western blotting (See, Examples. The synthetic coding sequences were assembled at Chiron Corp. or by the Midland Certified Reagent Company (Midland, Texas).

Exemplary modified coding sequences are presented as synthetic Env expression cassettes in Table 1A and 1B. The following expression cassettes (i) have unique, terminal *EcoRI* and *XbaI* cloning sites; (ii) include Kozak sequences

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to promote optimal translation; (iii) tPA signal sequences
(to direct the ENV polypeptide to the cell membrane, see,
e.g., Chapman et al., *infra*); (iv) open reading frames
optimized for expression in mammalian cells; and (v) a
5 translational stop signal codon.

Table 1A: Exemplary Synthetic Env Expression
Cassettes (SF162)

	Expression Cassette	Seq Id	Further Information
5	gp120 SF162	30	wild-type; Figure 16
	gp140 SF162	31	wild-type; Figure 17
	gp160 SF162	32	wild-type; Figure 18
	gp120.modSF162	33	none; Figure 19
	gp120.modSF162.delV2	34	deleted V2 loop; Figure 20
10	gp120.modSF162.delV1/V2	35	deleted V1 and V2; Figure 21
	gp140.modSF162	36	none; Figure 23
	gp140.modSF162.delV2	37	deleted V2 loop; Figure 24
	gp140.modSF162.delV1/V2	38	deleted V1 and V2; Figure 25
	gp140.mut.modSF162	39	mutated cleavage site; Fig. 26
15	gp140.mut.modSF162.delV2	40	deleted V2; mutated cleavage site; Figure 27
	gp140.mut.modSF162.delV1/V2	41	deleted V1 & V2; mutated cleavage site; Figure 28
	gp140.mut7.modSF162	42	mutated cleavage site; Fig. 29
	gp140.mut7.modSF162.delV2	43	mutated cleavage site; deleted V2; Figure 30
	gp140.mut7.modSF162.delV1/V2	44	mutated cleavage site; deleted V1 and V2; Figure 31
20	gp140.mut8.modSF162	45	mutated cleavage site; Fig. 32
	gp140.mut8.modSF162.delV2	46	mutated cleavage site; deleted V2; Figure 33
	gp140.mut8.modSF162.delV1/V2	47	mutated cleavage site; deleted V1 and V2; Figure 34
	gp160.modSF162	48	none; Figure 35
	gp160.modSF162.delV2	49	deleted V2 loop; Figure 36
25	gp160.modSF162.delV1/V2	50	deleted V1 & V2; Figure 37

Table 1B:
Exemplary Synthetic Env Expression Cassettes(US4)

Expression Cassette	Seq Id	Further Information
gp120 US4	51	wild-type; Figure 38
gp140 US4	52	wild-type; Figure 39
gp160 US4	53	wild-type; Figure 40
gp120.modUS4	54	none; Figure 41
gp120.modUS4.del 128-194	55	deletion in V1 and V2 regions; Figure 42
gp140.modUS4	56	none; Figure 43
gp140.mut.modUS4	57	mutated cleavage site; Figure 44
gp140TM.modUS4	58	native transmembrane region; Figure 45
gp140.modUS4.delV1/V2	59	deleted V1 and V2; Figure 46
gp140.modUS4.delV2	60	deleted V1; Figure 47
gp140.mut.modUS4.delV1/V2	61	mutated cleavage site; deleted V1 and V2; Figure 48
gp140.modUS4.del 128-194	62	deletion in V1 and V2 regions; Figure 49
gp140.mut.modUS4.del 128- 194	63	mutated cleavage site; deletion in V1 and V2 regions; Figure 50
gp160.modUS4	64	none; Figure 51
gp160.modUS4.delV1	65	deleted V1; Figure 52
gp160.modUS4.delV2	66	deleted V2; Figure 53
gp160.modUS4.delV1/V2	67	deleted V1 and V2; Figure 54
gp160.modUS4del 128-194	68	deletion in V1 and V2 regions; Figure 55

Alignments of the sequences presented in the above
tables are presented in Figures 66A and 66B.

A common region (Env-common) extends from nucleotide

position 1186 to nucleotide position 1329 (SEQ ID NO:69,
Fig. 56) relative to the wild-type US4 sequence and from
nucleotide position 1117 to position 1260 (SEQ ID NO:79,
Fig. 57) relative to the wild-type SF162 sequence. The
5 synthetic sequences of the present invention corresponding
to these regions are presented, as SEQ ID NO:71 (Figure 58)
for the synthetic Env US4 common region and as SEQ ID NO:72
(Figure 59) for the synthetic Env SF162 common region.

Percent identity to this sequence can be determined,
10 for example, using the Smith-Waterman search algorithm (Time
Logic, Incline Village, NV), with the following exemplary
parameters: weight matrix = nuc4x4hb; gap opening penalty =
20, gap extension penalty = 5, reporting threshold = 1;
alignment threshold = 20.

15 Various forms of the different embodiments of the
present invention (e.g., constructs) may be combined.

F. Cloning Synthetic Env Expression Cassettes of the
Present Invention.

20 The synthetic DNA fragments encoding the Env
polypeptides were typically cloned into the eucaryotic
expression vectors described above for Gag, for example,
pCMVKm2/pCMVlink (Figure 4), pCMV6a, pESN2dhfr (Figure 13A),
pCMVIII (Figure 13B; alternately designated as the pCMV-PL-
25 E-dhfr/neo vector).

Exemplary designations for pCMVlink vectors containing
synthetic expression cassettes of the present invention are
as follows: pCMVlink.gp140.modSF162; pCMVlink.gp140.-
modSF162.delV2; pCMVlink.gp140.mut.modSF162;
30 pCMVlink.gp140.mut.modSF162.delV2; pCMVKm2.gp140modUS4;
pCMVKm2.gp140.modUS4.delV2; pCMVKm2.gp140.mut.modUS4; and,

pCMVKm2.gp140.mut.modUS4.delV1/V2.

G. Generation of Synthetic Tat Expression Cassettes

Tat coding sequences have also been modified according to the teachings of the present specification. The wild type nucleotide sequence encoding tat from variant SF162 is presented in Figure 76 (SEQ ID NO:85). The corresponding wild-type amino acid sequence is presented in Figure 77 (SEQ ID NO:86). Figure 81 (SEQ ID NO:89) shows the nucleotide sequence encoding the amino terminal of the tat protein and the codon encoding cystein-22 is underlined. Other exemplary constructs encoding synthetic tat polypeptides are shown in Figures 78 and 79 (SEQ ID NOs:87 and 88). In one embodiment (SEQ ID NO:88), the cystein residue at position 22 is replaced by a glycine. Caputo et al. (1996) *Gene Therapy* 3:235 have shown that this mutation affects the trans activation domain of Tat.

Various forms of the different embodiments of the invention, described herein, may be combined.

H. Deposit of Vectors

Selected exemplary constructs shown below and described herein are deposited at Chiron Corporation, Emeryville, CA, 94662-8097, and were sent to the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 on December 27, 1999.

	Plasmid Name	Chiron Deposit #	Date Sent to ATCC
	pCMVgpl60.modUS4	5094	27 Dec 99
	pCMVgpl60delI.modUS4	5095	27 Dec 99
	pCMVgpl60del2.modUS4	5096	27 Dec 99
5	pCMVgpl60del-2.modUS4	5097	27 Dec 99
	pCMVgpl60del128-194.mod.US4	5098	27 Dec 99
	pCMVgpl40mut.modUS4del128-194	5100	27 Dec 99
	pCMVgpl40.mut.mod.US	5101	27 Dec 99
	pCMVgpl60.modSF162	5125	27 Dec 99
10	pCMVgpl60.modSF162.delV2	5126	27 Dec 99
	pCMVgpl60.modSF162.delV1V2	5127	27 Dec 99
	pCMVgpl40.mut.modSF162delV2	5128	27 Dec 99
	pCMVgpl40.mut7.modSF162	5129	27 Dec 99
	pCMVgpl40.mut7.modSF162delV2	5130	27 Dec 99
15	pCMVgpl40.mut8.modSF162	5131	27 Dec 99
	pCMVgpl40.mut8.modSF162delV2	5132	27 Dec 99
	pCMVgpl40.mut8.modSF162delV1V2	5133	27 Dec 99
	pCMVKm2.Gagprot.Mod.SF2.GP1	5150	27 Dec 99
	pCMVKm2.Gagprot.Mod.SF2.GP2	5151	27 Dec 99
20			

Example 2

Expression Assays for the

Synthetic Gag, Env and Tat Coding Sequences

25 A. Gag and Gag-Protease Coding Sequences

The HIV-1SF2 wild-type Gag (SEQ ID NO:1) and Gag-protease (SEQ ID NO:2) sequences were cloned into expression vectors having the same features as the vectors into which

the synthetic Gag (SEQ ID NO:4) and Gag-protease (SEQ ID NOs:5, 78 or 79)) sequences were cloned.

Expression efficiencies for various vectors carrying the HIV-1SF2 wild-type and synthetic Gag sequences were evaluated as follows. Cells from several mammalian cell lines (293, RD, COS-7, and CHO; all obtained from the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209) were transfected with 2 μ g of DNA in transfection reagent LT1 (PanVera Corporation, 545 Science Dr., Madison, WI). The cells were incubated for 5 hours in reduced serum medium (Opti-MEM, Gibco-BRL, Gaithersburg, MD). The medium was then replaced with normal medium as follows: 293 cells, IMDM, 10% fetal calf serum, 2% glutamine (BioWhittaker, Walkersville, MD); RD and COS-7 cells, D-MEM, 10% fetal calf serum, 2% glutamine (Opti-MEM, Gibco-BRL, Gaithersburg, MD); and CHO cells, Ham's F-12, 10% fetal calf serum, 2% glutamine (Opti-MEM, Gibco-BRL, Gaithersburg, MD). The cells were incubated for either 48 or 60 hours. Supernatants were harvested and filtered through 0.45 μ m syringe filters and, optionally, stored at -20°C.

Supernatants were evaluated using the Coulter p24-assay (Coulter Corporation, Hialeah, FL, US), using 96-well plates coated with a murine monoclonal antibody directed against HIV core antigen. The HIV-1 p24 antigen binds to the coated wells. Biotinylated antibodies against HIV recognize the bound p24 antigen. Conjugated strepavidin-horseradish peroxidase reacts with the biotin. Color develops from the reaction of peroxidase with TMB substrate. The reaction is terminated by addition of 4N H₂SO₄. The intensity of the color is directly proportional to the amount of HIV p24 antigen in a sample.

The results of these expression assays are presented in
Tables 2A and 2B. Tables 2A and 2B shows data obtained
using the synthetic Gag-protease expression cassette of SEQ
ID NO:5. Similar results were obtained using the Gag-
5 protease expression cassettes of SEQ ID NOs:78 and 79.

Table 2: in vitro gag and gagprot p24 expression

5 TABLE 2a. Increased in vitro expression from modified vs. native gag plasmids in supernatants and lysates from transiently transfected cells

experiment	native (nat) ^a modified (mod) ^b	supernatant (sup) lysate (lys)	cell line	hours post transfection	total ng p24 (fold increase)
1	nat	sup	293	48	3.4
	mod	sup	293	48	1260 (371)
	nat	sup	293	60	3.2
	mod	sup	293	60	2222 (694)
2	nat	sup	293	60	1.8
	mod	sup	293	60	1740 (966)
3	nat	sup	293	60	1.8
	mod	sup	293	60	580 (322)
4	nat	lys	293	60	1.5
	mod	lys	293	60	85 (57)
1	nat	sup	RD	48	5.6
	mod	sup	RD	48	66 (12)
	nat	sup	RD	60	7.8
	mod	sup	RD	60	70.2 (9)
2	nat	lys	RD	60	1.9
	mod	lys	RD	60	7.8 (4)
1	nat	sup	COS-7	48	0.4
	mod	sup	COS-7	48	33.4 (84)
2	nat	sup	COS-7	48	0.4
	mod	sup	COS-7	48	10 (25)
	nat	lys	COS-7	48	3
	mod	lys	COS-7	48	14 (5)

^a pCMVLink.Gag.SF2.PRE

^b pCMVKm2.GagMod.SF2

5

TABLE 2b. *In vitro* expression from modified gag and gagprotease plasmids in supernatants and lysates from transiently transfected cells

plasmid	supernatant (sup) lysate (lys)	cell line	hours post transfection	total ng p24 ^d
Gag ^a	sup	293	60	760
GagProt(GP1) ^b	sup	293	60	380
GagProt(GP2) ^c	sup	293	60	320
Gag	lys	293	60	78
GagProt(GP1)	lys	293	60	1250
GagProt(GP2)	lys	293	60	400
Gag	sup	COS-7	72	40
GagProt(GP1)	sup	COS-7	72	150
GagProt(GP2)	sup	COS-7	72	290
Gag	lys	COS-7	72	60
GagProt(GP1)	lys	COS-7	72	63
GagProt(GP2)	lys	COS-7	72	58

^a pCMVKm2.GagMod.SF2

^b pCMVKm2.GagProtMod.SF2(GP1) *gagprotease* with codon optimization and inactivation of INS in *protease*

^c pCMVKm2.GagProtMod.SF2(GP2) *gagprotease* with only inactivation of INS in *protease*

^d Shown are representative results from 3 independent experiments for each cell line tested.

The data showed that the synthetic Gag and Gag-protease expression cassettes provided dramatic increases in production of their protein products, relative to the native (HIV-1SF2 wild-type) sequences, when expressed in a variety of cell lines.

B. Env Coding Sequences

The HIV-SF162 ("SF162") wild-type Env (SEQ ID NO:1-3) and HIV-US4 ("US4") wild-type Env (SEQ ID NO:22-24) sequences were cloned into expression vectors having the same features as the vectors into which the synthetic Env sequences were cloned.

Expression efficiencies for various vectors carrying the SF162 and US4 wild-type and synthetic Env sequences were evaluated essentially as described above for Gag except that cell lysates were prepared in 40 μ l lysis buffer (1.0 % NP40, 0.1 M Tris pH 7.5) and frozen at -20°C and capture ELISAs were performed as follows.

For Capture ELISAs, 250 ng of an ammonium sulfate IgG cut of goat polyclonal antibody to gp120SF2/env2-3 was used to coat each well of a 96-well plate (Corning, Corning, NY). Serial dilutions of gp120/SF2 protein (MID 167) were used to set the quantitation curve from which expression of US4 or SF162 gp120 proteins from transfection supernatant and lysates were calculated. Samples were screened undiluted and, optionally, by serial 2-fold dilutions. A human polyclonal antibody to HIV-1 gp120/SF2 was used to detect bound gp120 envelope protein, followed by horse-radish peroxidase (HRP)-labeled goat anti-human IgG conjugates. TMB (Pierce, Rockford, IL) was used as the substrate and the reaction is terminated by addition of 4N H₂SO₄. The

reaction was quantified by measuring the optical density
(OD) at 450 nm. The intensity of the color is directly
proportional to the amount of HIV gp120 antigen in a sample.
Purified SF2 gp120 protein was diluted and used as a
5 standard.

The results of the transient expression assays are
presented in Tables 3 and 4. Table 3 depicts transient
expression in 293 cells transfected with a pCMVKm2 vector
carrying the Env cassette of interest. Table 4 depicts
10 transient expression in RD cells transfected with a pCMVKm2
vector carrying the Env cassette of interest.

Table 3

Native (N) Synthetic (S)	Cell Line	Total sup (ng)	Sup fold increase (S v. N)	Total cell lysate (ng)	Cell lysate fold increase (S v. N)	Total (ng)	Total fold increase (S v. N)
N-gp120.US4	RD	87		<1		88	
S-gp120.modUS4	RD	690	8	2	5	693	8
N-gp140.US4	RD	526		0		526	
S-gp140.modUS4	RD	1305	2	1	2	1306	2
S-gp140mut.modUS4	RD	35	N/A	25	N/A	60	N/A
S-gp140TM.modUS4	RD	0	N/A	5	N/A	5	N/A
N-gp160.US4	RD	0		8		8	
S-gp160.modUS4	RD	0	0	30	4	30	4

Table 4

CHO Cell Lines Expression Level of US4 Envelope Constructs			
Constructs	CHO Clone #	MTX Level	Expression Level* (ng/ml)
gp120.modUS4	1	3.2 μ M	250-450
	2	1.6 μ M	350-450
	3	200nM	230-580
	4	200nM	300-500
gp140.modUS4	1	1 μ M	155-300
	2	1 μ M	100-260
	3	1 μ M	200-430
gp140.mut. modUS4	1	1 μ M	110-270
	2	1 μ M	100-235
	3	1 μ M	100-220
gp140.modUS4 .delV1/V2	1	50nM	313-587**
	2	50nM	237-667**
	3	50nM	492-527**
gp140.mut. modUS4.delV1 /V2	1	50nM	46-328**
	2	50nM	82-318**
	3	50nM	204-385**

*All samples measured at T-75 flask stage unless otherwise indicated

**at 24 well and 6 well plate stages

***in a three liter bioreactor perfusion culture this clone yielded approximately 2-5 μ g/ml.

The data showed that the synthetic Env and expression cassettes provided a significant increase in production of their protein products, relative to the native (HIV-1SF162 or US4 wild-type) sequences, when expressed in a variety of cell lines.

C. CHO Cell line Env expression data

Chinese hamster ovary (CHO) cells were transfected with plasmid DNA encoding the synthetic HIV-1 gp120 or gp140 proteins (e.g., pESN2dhfr or pCMVIII vector backbone) using Mirus TransIT-LT1 polyamine transfection reagent (Pan Vera) according to the manufacturers instructions and incubated for 96 hours. After 96 hours, media was changed to selective media (F12 special with 250 μ g/ml G418) and cells were split 1:5 and incubated for an additional 48 hours. Media was changed every 5-7 days until colonies started forming at which time the colonies were picked, plated into 96 well plates and screened by gp120 Capture ELISA. Positive clones were expanded in 24 well plates and screened several times for Env protein production by Capture ELISA, as described above. After reaching confluency in 24 well plates, positive clones were expanded to T25 flasks (Corning, Corning, NY). These were screened several times after confluency and positive clones were expanded to T75 flasks.

Positive T75 clones were frozen in LN2 and the highest expressing clones amplified with 0-5 μ M methotrexate (MTX) at several concentrations and plated in 100mm culture dishes. Plates were screened for colony formation and all positive clones were again expanded as described above. Clones were expanded and amplified and screened at each step by gp120

capture ELISA. Positive clones were frozen at each methotrexate level. Highest producing clones were grown in perfusion bioreactors (3L, 100L) for expansion and adaptation to low serum suspension culture conditions for
5 scale-up to larger bioreactors.

Tables 5 and 6 show Capture ELISA data from CHO cells transfected with pCMVIII vector carrying a cassette encoding synthetic HIV-US4 and SF162 Env polypeptides (e.g., mutated cleavage sites, modified codon usage and/or deleted
10 hypervariable regions). Thus, stably transfected CHO cell lines which express Env polypeptides (e.g., gp120, gp140-monomeric, and gp140-oligomeric) have been produced.

Table 5

CHO Cell Lines Expression Level of US4 Envelope Constructs			
Constructs	CHO Clone #	MTX Level	Expression Level* (ng/ml)
gp120.modUS4	1	3.2 μ M	250-450
	2	1.6 μ M	350-450
	3	200nM	230-580***
	4	200nM	300-500
gp140.modUS4	1	1 μ M	155-300
	2	1 μ M	100-260
	3	1 μ M	200-430
gp140.mut. modUS4	1	1 μ M	110-270
	2	1 μ M	100-235
	3	1 μ M	100-220
gp140.modUS4 .delV1/V2	1	50nM	313-587**
	2	50nM	237-667**
	3	50nM	492-527**
gp140.mut. modUS4.delV1 /V2	1	50nM	46-328**
	2	50nM	82-318**
	3	50nM	204-385**

*All samples measured at T-75 flask stage unless otherwise indicated

**at 24 well and 6 well plate stages

***in a three liter bioreactor perfusion culture this clone yielded approximately 2-5 μ g/ml.

Table 6

CHO Cell Lines Expression Level of SF162 Envelope Constructs			
Constructs	CHO Clone #	MTX Level	Expression Level* (ng/ml)
gp120.modSF162	1	0	755-2705
	2	0	928-1538
	3	0	538-1609
gp140.modSF162	1	20 nM	180-350
gp140.mut. modSF162	1	20 nM	164-451
	2	20 nM	188-487
	3	20 nM	233-804
gp120.modSF162 .delV2	1	800nM	528-1560
	2	800nM	487-1878
	3	800nM	589-1212
gp140.modSF162 .delV2	1	800nM	300-600
	2	800nM	200-400
	3	800nM	200-500
gp140.mut. modSF162.delV2	1	800nM	300-700
	2	400nM	1161
	3	800nM	400-600
	4	400nM	1600-2176

*All samples measured at T-75 flask stage unless otherwise indicated

The results presented above demonstrate the ability of the constructs of the present invention to provide expression of Env polypeptides in CHO cells. Production of polypeptides using CHO cells provides (i) correct

glycosylation patterns and protein conformation (as determined by binding to panel of MAbs); (ii) correct binding to CD4 receptor molecules; (iii) absence of non-mammalian cell contaminants (e.g., insect viruses and/or cells); and (iv) ease of purification.

D. Tat Coding Sequences

The HIV-SF162 ("SF162") wild-type Tat (SEQ ID NO:85) sequences were cloned into expression vectors having the same features as the vectors into which the synthetic Tat sequences were cloned (SEQ ID NOs:87, 88 and 89).

Expression efficiencies for various vectors carrying the SF162 wild-type and synthetic Tat sequences are evaluated essentially as described above for Gag and Env using capture ELISAs with the appropriate anti-tat antibodies and/or CHO cell assays. Expression of the polypeptides encoded by the synthetic cassettes is improved relative to wild type.

Example 3

Western Blot Analysis of Expression

A. Gag and Gag-Protease Coding Sequences

Human 293 cells were transfected as described in Example 2 with pCMV6a-based vectors containing native or synthetic Gag expression cassettes. Cells were cultivated for 60 hours post-transfection. Supernatants were prepared as described. Cell lysates were prepared as follows. The cells were washed once with phosphate-buffered saline, lysed with detergent [1% NP40 (Sigma Chemical Co., St. Louis, MO) in 0.1 M Tris-HCl, pH 7.5], and the lysate transferred into fresh tubes. SDS-polyacrylamide gels (pre-cast 8-16%;

Novex, San Diego, CA) were loaded with 20 μ l of supernatant or 12.5 μ l of cell lysate. A protein standard was also loaded (5 μ l, broad size range standard; BioRad Laboratories, Hercules, CA). Electrophoresis was carried
5 out and the proteins were transferred using a BioRad Transfer Chamber (BioRad Laboratories, Hercules, CA) to Immobilon P membranes (Millipore Corp., Bedford, MA) using the transfer buffer recommended by the manufacturer (Millipore), where the transfer was performed at 100 volts
10 for 90 minutes. The membranes were exposed to HIV-1-positive human patient serum and immunostained using o-phenylenediamine dihydrochloride (OPD; Sigma).

The results of the immunoblotting analysis showed that cells containing the synthetic Gag expression cassette
15 produced the expected p55 protein at higher per-cell concentrations than cells containing the native expression cassette. The Gag p55 protein was seen in both cell lysates and supernatants. The levels of production were significantly higher in cell supernatants for cells
20 transfected with the synthetic Gag expression cassette of the present invention. Experiments performed in support of the present invention suggest that cells containing the synthetic Gag-prot expression cassette produced the expected Gag-prot protein at comparably higher per-cell
25 concentrations than cells containing the native expression cassette.

In addition, supernatants from the transfected 293 cells were fractionated on sucrose gradients. Aliquots of the supernatant were transferred to Polyclear™ ultra-
30 centrifuge tubes (Beckman Instruments, Columbia, MD), underlaid with a solution of 20% (wt/wt) sucrose, and subjected to 2 hours centrifugation at 28,000 rpm in a Beckman SW28

rotor. The resulting pellet was suspended in PBS and layered onto a 20-60% (wt/wt) sucrose gradient and subjected to 2 hours centrifugation at 40,000 rpm in a Beckman SW41ti rotor.

5 The gradient was then fractionated into approximately 10 x 1 ml aliquots (starting at the top, 20%-end, of the gradient). Samples were taken from fractions 1-9 and were electrophoresed on 8-16% SDS polyacrylamide gels. Fraction number 4 (the peak fraction) corresponds to the expected
10 density of Gag protein VLPs. The supernatants from 293/synthetic Gag cells gave much stronger p55 bands than supernatants from 293/native Gag cells, and, as expected, the highest concentration of p55 in either supernatant was found in fraction 4.

15 These results demonstrate that the synthetic Gag expression cassette provides superior production of both p55 protein and VLPs, relative to the native Gag coding sequences.

20 B. Env Coding Sequences

 Human 293 cells were transfected as described in Example 2 with pCMVKm2-based; pCMVlink-based; p-CMVII-based or pESN2-based vectors containing native or synthetic Env expression cassettes. Cells were cultivated for 48 or 60
25 hours post-transfection. Cell lysates and supernatants were prepared as described (Example 2). Briefly, the cells were washed once with phosphate-buffered saline, lysed with detergent [1% NP40 (Sigma Chemical Co., St. Louis, MO)] in 0.1 M Tris-HCl, pH 7.5], and the lysate transferred into
30 fresh tubes. SDS-polyacrylamide gels (pre-cast 8-16%; Novex, San Diego, CA) were loaded with 20 μ l of supernatant or 12.5 μ l of cell lysate. A protein molecular weight

standard and an HIV SF2 gp120 positive control protein (5 μ l, broad size range standard; BioRad Laboratories, Hercules, CA) were also loaded. Electrophoresis was carried out and the proteins were transferred using a BioRad Transfer Chamber (BioRad Laboratories, Hercules, CA) to Immobilon P membranes (Millipore Corp., Bedford, MA) using the transfer buffer recommended by the manufacturer (Millipore), where the transfer was performed at 100 volts for 90 minutes. The membranes were then reacted against polyclonal goat anti-gp120SF2/env2-3 anti-sera, followed by incubation with swine anti-goat IgG-peroxidase (POD) (Sigma, St. Louis, MO). Bands indicative of binding were visualized by adding DAB with hydrogen peroxide which deposits a brown precipitate on the membranes.

The results of the immunoblotting analysis showed that cells containing the synthetic Env expression cassette produced the expected Env gp proteins of the predicted molecular weights as determined by mobilities in SDS-polyacrylamide gels at higher per-cell concentrations than cells containing the native expression cassette. The Env proteins were seen in both cell lysates and supernatants. The levels of production were significantly higher in cell supernatants for cells transfected with the synthetic Env expression cassette of the present invention.

C. Tat Coding Sequences

Human 293 cells are transfected as described in Example 2 with various vectors containing native or synthetic Tat expression cassettes. Cells are cultivated and isolated proteins analyzed as described above. Immunoblotting analysis shows that cells containing the synthetic Tat

expression cassette produced the expected Tat proteins of the predicted molecular weights as determined by mobilities in SDS-polyacrylamide gels at higher per-cell concentrations than cells containing the native expression cassette.

5

Example 4

Purification of Env polypeptides

A. Purification of Oligomeric gp140

Purification of oligomeric gp140 (o-gp140 US4) was
10 conducted essentially as shown in Figure 60. For the
experiments described herein, o-gp140 refers to oligomeric
gp140 in either native or modified (e.g., optimized
expression sequences, deleted, mutated, truncated, etc.)
form. Briefly, concentrated (30-50X) supernatants obtained
15 from CHO cell cultures were loaded onto an anion exchange
(DEAE) column which removed DNA and other serum proteins.
The eluted material was loaded onto a ceramic hydroxyapatite
column (CHAP) which bound serum proteins but not HIV Env
proteins. The flow-through from the DEAE and CHAP columns
20 was loaded onto a Protein A column as a precautionary step
to remove any remaining serum immunoglobulins. The Env
proteins in the flow-through were then captured using the
lectin gluvanthus navalis (GNA, Vector Labs, Burlingame,
CA). GNA has high affinity for mannose rich carbohydrates
25 such as Env. The Env proteins were then eluted with GNA
substrate. To remove other highly glycosylated proteins, a
cation exchange column (SP) was used to purify gp140/gp120.
In a final step, which separates gp120 from o-gp140, a gel
filtration column was used to separate oligomers from
30 monomers. Sizing and chromatography analysis of the final

product revealed that this strategy lead to the successful isolation of oligomeric gp140.

B. Purification of gp120

5 Purification of gp120 was conducted essentially as previously described for other Env proteins. Briefly, concentrated supernatants obtained from CHO cell cultures were loaded onto an anion exchange (DEAE) column which removed DNA and other serum proteins. The eluted material
10 was loaded onto a ceramic hydroxyapatite column (CHAP) which bound serum proteins but not HIV Env proteins. The flow-through from the CHAP column was loaded a cation exchange column (SP) where the flow-through was discarded and the bound fraction eluted with salt. The eluted fraction(s)
15 were loaded onto a Suprose 12/Superdex 200 Tandem column (Pharmacia-Upjohn, Uppsala, Sweden) from which purified gp120 was obtained. Sizing and chromatography analysis of the final product revealed that this strategy successfully purified gp120 proteins.

20

Example 5

Analysis of Purified Env Polypeptides

A. Analysis of o-gp140

25 It is well documented that HIV Env protein binds to CD4 only in its correct conformation. Accordingly, the ability of o-gp140 US4 polypeptides, produced and purified as described above, to bind CD4 cells was tested. O-gp140 US4 was incubated for 15 minutes with FITC-labeled CD4 at room temperature and loaded onto a Biosil 250 (BioRad) size
30 exclusion column using Waters HPLC. CD4-FITC has the longest retention time (2.67 minutes), followed by CD4-FITC-gp120 (2.167 min). The shortest retention time (1.9 min) was

observed for CD4-FITC-o-gp140 US4 indicating that, as expected, o-gp140 US4 binds to CD4 forming a large complex which reduces retention time on the column. Thus, the o-gp140 US4 produced and purified as described above is of the correct size and conformation.

In addition, the US4 o-gp140, purified as described above, was also tested for its ability to bind to a variety of monoclonal antibodies with known epitope specificities for the CD4 binding site, the CD4 inducible site, the V3 loop and oligomer-specific gp41 epitope. O-gp140 bound strongly to these antibodies, indicating that the purified protein retains its structural integrity.

B. Analysis of gp120

As described above, CD4-FITC binds gp120, as demonstrated by the decreased retention time on the HPLC column. Thus, US4 gp120 purified by the above method retains its conformational integrity. In addition, the properties of purified gp120 can be tested by examining its integrity and identity on western blots, as well as, by examining protein concentration, pH, conductivity, endotoxin levels, bioburden and the like. US4 gp120, purified as described above, was also tested for its ability to bind to a variety of monoclonal antibodies with known epitope specificities for the CD4 binding site, the CD4 inducible site, the V3 loop and oligomer-specific gp41 epitope. The pattern of mAb binding to gp120 indicated that the purified protein retained its structural integrity, for example, the purified gp120 did not bind the mAb having the oligomer-specific gp41 epitope (as expected).

Example 6

Electron Microscopic Evaluation of VLP Production

The cells for electron microscopy were plated at a density of 50-70% confluence, one day before transfection.

5 The cells were transfected with 10 μ g of DNA using transfection reagent LT1 (Panvera) and incubated for 5 hours in serum-reduced medium (see Example 2). The medium was then replaced with normal medium (see Example 2) and the cells were incubated for 14 hours (COS-7) or 40 hours (CHO).
10 After incubation the cells were washed twice with PBS and fixed with 2% glutaraldehyde. Electron microscopy was performed by Prof. T.S. Benedict Yen, Veterans Affairs, Medical Center, San Francisco, CA).

Electron microscopy was carried out using a
15 transmission electron microscope (Zeiss 10c). The cells were pre-stained with osmium and stained with uranium acetate and lead citrate. The magnification was 100,000X.

Figures 3A and 3B show micrographs of CHO cells transfected with pCMVKM2 carrying the synthetic Gag
20 expression cassette (SEQ ID NO:5) or carrying the Gag-prot expression cassette (SEQ ID NO:79). In the figure, free and budding immature virus-like-particles (VLP) of the expected size (100 nm) are seen for the Gag expression cassette (Figure 3A) and both immature and mature VLPs are seen for
25 the Gag-prot expression cassette (Figure 3B). COS-7 cells transfected with the same vector have the same expression pattern. VLP can also be found intracellularly in CHO and COS-7 cells.

Native and synthetic Gag expression cassettes were
30 compared for their associated levels of VLP production when used to transfect human 293 cells. The comparison was performed by density gradient ultracentrifugation of cell

supernatants and Western-blot analysis of the gradient fractions. There was a clear improvement in production of VLPs when using the synthetic Gag construct.

5

Example 7

Expression of Virus-like Particles in the Baculovirus System

A. Expression of Native HIV p55 Gag

To construct the native HIV p55 Gag baculovirus shuttle vector, the prototype SF2 HIV p55 plasmid, pTM1-Gag (Selby
10 M.J., et al., *J Virol.* 71(10):7827-7831, 1997), was digested with restriction endonucleases *Nco*I and *Bam*HI to extract a 1.5 Kb fragment that was subsequently subcloned into pAcC4 (*Bio/Technology* 6:47-55, 1988), a derivative of pAc436. Generation of the recombinant baculovirus was achieved by
15 co-transfecting 2 μ g of the HIV p55 Gag pAcC4 shuttle vector with 0.5 μ g of linearized, *Autographa californica* baculovirus (AcNPV) wild-type viral DNA into *Spodoptera frugiperda* (Sf9) cells (Kitts, P.A., Ayres M.D., and Possee R.D., *Nucleic Acids Res.* 18:5667-5672, 1990). The isolation
20 of recombinant virus expressing HIV p55 Gag was performed according to standard techniques (O'Reilly, D.R., L.K. Miller, and V. A. Luckow, *Baculovirus Expression Vector: A Laboratory Manual*, W.H. Freeman and Company, New York, 1992).

25 Expression of the HIV p55 Gag was achieved using a 500 ml suspension culture of Sf9 cells grown in serum-free medium (Miaorella, B., D. Inlow, A. Shauger, and D. Harano, *Bio/Technology* 6:1506-1510, 1988) that had been infected with the HIV p55 Gag recombinant baculovirus at a
30 multiplicity of infection (MOI) of 10. Forty-eight hours post-infection, the supernatant was separated by

centrifugation and filtered through a 0.2 μ m filter. Aliquots of the supernatant were then transferred to Polyclar™ (Beckman Instruments, Palo Alto, CA) ultracentrifuge tubes, underlaid with 20% (wt/wt) sucrose, and subjected to 2 hours centrifugation at 24,00 rpm using a Beckman SW28 rotor.

The resulting pellet was suspended in Tris buffer (20 mM Tris HCl, pH 7.5, 250 mM NaCl, and 2.5 mM ethylenediaminetetraacetic acid [EDTA]), layered onto a 20-60% (wt/wt) sucrose gradient, and subjected to 2 hours centrifugation at 40,000 rpm using a Beckman SW41ti rotor. The gradient was then fractionated starting at the top (20% sucrose) of the gradient into approximately twelve 0.75 ml aliquots. A sample of each fraction was electrophoresed on 8-16% SDS polyacrylamide gels and the resulting bands were visualized after commassie staining (Figure 4). Additional aliquots were subjected to refractive index analysis.

The results shown in Figure 4 indicated that the p55 Gag virus-like particles banded at a sucrose density of range of 1.15 - 1.19 g/ml with the peak at approximately 1.17 g/ml. The peak fractions were pooled and concentrated by a second 20% sucrose pelleting. The resulting pellet was suspended in 1 ml of Tris buffer (described above). The total protein yield as estimated by Bicimchrominic Acid (BCA) (Pierce Chemical, Rockford, IL) was 1.6 mg.

B. Expression of Synthetic HIV p55 Gag

A baculovirus shuttle vector containing the synthetic p55 Gag sequence was constructed as follows. The synthetic HIV p55 expression cassette (Example 1) was digested with restriction enzyme *Sal*I followed by incubation with T4-DNA

polymerase. The resulting fragment was isolated (PCR Clean-Up™, Promega, Madison, WI) and then digested with *Bam*HI endonuclease. The shuttle vector pAcCl3 (Munemitsu S., et al., *Mol Cell Biol.* 10(11):5977-5982, 1990) was linearized
5 by digestion with *Eco*I, followed by incubation with T4-DNA polymerase, and then isolated (PCR Clean-Up™). The linearized vector was digested with *Bam*HI, treated with alkaline phosphatase, and isolated by size fragmentation in an agarose gel. The isolated 1.5 kb fragment was ligated
10 with the prepared pAcCl3 vector. The resulting clone was designated pAcCl3-Modif.p55Gag.

The expression conditions for the synthetic HIV p55 VLPs differed from those of the native p55 Gag as follows: a culture volume of 1 liter used instead of 500 ml;
15 *Trichoplusia ni* (Tn5) (Wickham, T.J., and Nermerow, G.R., *BioTechnology Progress*, 9:25-30, 1993) insect cells were used instead of Sf9 insect cells; and, an MOI of 3 was instead of an MOI of 10. Experiments performed in support of the present invention showed that there was no
20 appreciable difference in expression level between the Sf9 and Tn5 insect cells with the native p55 clone. In terms of MOI, experience with the native p55 clone suggested that an MOI of 10 resulted in higher expression (approximately 2-fold) of VLPs than a lower MOI.

25 The sucrose pelleting and banding methods used for the synthetic p55 VLPs were similar to those employed for the native p55 VLPs (described above), with the following exceptions: pelleted VLPs were suspended in 4 ml of phosphate buffered saline (PBS) instead of 1.0 ml of the
30 Tris buffer; and four, 20-60% sucrose gradients were used instead of a single gradient. Also, due to the high

concentration of banded VLPs, further concentration by pelleting was not required. The peak fractions from all 4 gradients were simply dialyzed against PBS. The approximate density of the banded VLPs ranged from 1.23-1.28 g/ml. A
5 total protein yield as estimated by BCA was 46 mg. Results from the sucrose gradient banding of the synthetic p55 are shown in Figure 5.

A comparison of the total amount of purified HIV p55 Gag from several preparations obtained from the two
10 baculovirus expression cassettes has been summarized in Figure 6. The average yield from the native p55 was 3.16 mg/liter of culture (n=5, standard deviation (sd) ± 1.07 , range = 1.8-4.8 mg/L) whereas the average yield from the synthetic p55 was more than ten-fold higher at 44.5 mg/liter
15 of culture (n=2, sd= ± 6.4).

In addition to a higher total protein yield, the final product from the synthetic p55-expressed Gag consistently contained lower amounts of contaminating baculovirus proteins than the final product from the native p55-
20 expressed Gag. This difference can be seen in the two commassie-stained gels Figures 4 and 5.

C. Expression of Native and Synthetic Gag-Core

Expression of the HIV p55 Gag/HCV Core 173 (SEQ ID
25 NO:8) was achieved using a 2.5 liter suspension culture of Sf9 cells grown in serum-free medium (Miaorella, B., D. Inlow, A. Shauger, and D. Harano. 1988 Bio/Technology 6:1506-1510). The cells were infected with an HIV p55 Gag/HCV Core 173 recombinant baculovirus. Forty-eight hours
30 post-infection, the supernatant was separated from the cells by centrifugation and filtered through a 0.2 μ m filter.

660621 9192460

Aliquots of the supernatant were then transferred to a Polyclear™ (Beckman Instruments, Palo Alto, CA) ultracentrifuge tubes containing 30% (wt/wt) sucrose, and subjected to 2 hours of centrifugation at 24,000 rpm in a
5 Beckman SW28 rotor and ultracentrifuge.

The resulting pellet was suspended in Tris buffer (50 mM Tris-HCl, pH 7.5, 500 mM NaCl) and layered onto a 30-60% (wt/wt) sucrose gradient and subjected to 2 hours centrifugation at 40,000 rpm in a Beckman SW41ti rotor and
10 ultracentrifuge. The gradient was then fractionated starting at the top (30%) of the gradient into approximately 11 x 1.0 ml aliquots. A sample of each fraction was electrophoresed on 8-16% SDS polyacrylamide gels and the resulting bands were visualized after commassie staining.

15 A subset of aliquots were also subjected to Western blot analysis using monoclonal antibody 76C.5EG (Steimer, K.S., et al., *Virology* 150:283-290, 1986) which is specific for HIV p24 (a subunit of HIV p55). The peak fractions from the sucrose gradient were pooled and concentrated by a
20 second 20% sucrose pelleting. The resulting pellet was suspended in 1 ml of buffer Tris buffer and the total protein yield as estimated by BCA (Pierce Chemical, Rockford, IL) was ~ 1.0 mg.

The results from the SDS PAGE are shown in Figure 8 and
25 the anti- p24 Western blot results are shown in Figure 9. Taken together, these results indicate that the HIV p55 Gag/HCV Core 173 chimeric VLPs banded at a sucrose density similar to that of the HIV p55 Gag VLPs and the visible protein band that migrated at a molecular weight of ~ 72,000
30 kd was reactive with the HIV p24-specific monoclonal antibody. An additional immunoreactive band at

approximately 55,000 kd also appeared to be reactive with the anti-p24 antibody and may be a degradation product.

Although aliquots from the above preparation were not tested for reactivity with an HCV Core-specific antibody (an anti-CD22 rabbit serum), results from a similar preparation are shown in Figure 10 and indicate that the main HCV Core-specific reactivity migrates at an approximate molecular weight of 72,000 kd which is in accordance with the predicted molecular weight of the chimeric protein.

The expression conditions for the synthetic HIV p55 Gag/HCV Core 173 (SEQ ID NO:8) VLPs differed from those of the native p55 Gag and are as follows: a culture volume of 1 liter used instead of 2.5 liters, *Trichoplusia ni* (Tn5) (Wickham, T.J., and Nemerow, G.R. 1993 BioTechnology Progress, 9:25-30) insect cells were used instead of Sf9 insect cells and an MOI of 3 was instead of an MOI of 10. The sucrose pelleting and banding methods used for the synthetic HIV p55 Gag/HCV Core 173 VLPs were similar to those employed for the native HIV p55 Gag/HCV Core 173 VLPs. However, differences included: pelleted VLPs were suspended in 1 ml of phosphate buffered saline (PBS) instead of 1.0 ml of the Tris buffer, and a single 20-60% sucrose gradients was used. A comparison of the total amount of purified HIV p55 Gag/HCV Core 173 from multiple preparations obtained from the two baculovirus expression cassettes showed that there was an increase in expression using the synthetic HIV p55 Gag/HCV Core 173 cassette.

D. Alternative method for the enrichment of HIV p55 Gag VLPs

In addition to purification from the media, p55 (Gag protein) expressed in baculovirus (e.g., using a synthetic expression cassette of the present invention) can also be purified as virus-like particles from the infected insect cells. For example, forty-eight hours post infection, the media and cell pellet are separated by centrifugation and the cell pellet is stored at -70°C until future use. At the time of processing, the cell pellet is suspended in 5 volumes of hypotonic lysis buffer (20 mM Tris-HCl, pH 8.2, 1 mM EGTA; 1 mM MgCl, and Complete Protease Inhibitor® (Boehringer Mannheim Corp., Indianapolis, IN]). If needed, the cells are then dounced 8-10 times to complete cell lysis.

The lysate is then centrifuged at approximately 1000-1500 x g for 20 minutes. The supernatant is decanted into UltraClear™ tubes, underlayered with 20% sucrose (w/w) and centrifuged at 24,000 rpm in SW28 buckets for 2 hours. The resulting pellet is suspended in Tris buffer (20 mM Tris HCl, pH 7.5, 250 mM NaCl, and 2.5 mM ethylene-diamine-tetraacetic acid (EDTA) with 0.1% IGEPAL detergent (Sigma Chemical, St. Louis, MO) and 250 units/ml of benzonase (American International Chemical, Inc., Natick, MA) and incubated at 4°C for at least 30 minutes. The suspension is subsequently layered onto a 20-60% sucrose gradient and spun at 40,000 rpm using an SW41ti rotor for 20-24 hours.

After ultracentrifugation, the sucrose gradient is fractionated and aliquots run on SDS PAGE to identify peak fractions. The peak fractions are dialyzed against PBS and measured for protein content. Negatively stained electron
5 mircographs typically show non-enveloped VLPs somewhat smaller in diameter (80-120 nm) than the budded VLPs. HIV Gag VLPs prepared in this manner are also capable of generating Gag-specific CTL responses in mice.

10 Example 8

In Vivo Immunogenicity of Synthetic Gag Expression Cassettes

A. Immunization

To evaluate the possibly improved immunogenicity of the synthetic Gag expression cassettes, a mouse study was
15 performed. The plasmid DNA, pCMVKM2 carrying the synthetic Gag expression cassette, was diluted to the following final concentrations in a total injection volume of 100 μ l: 20 μ g, 2 μ g, 0.2 μ g, and 0.02 μ g. To overcome possible negative dilution effects of the diluted DNA, the total DNA
20 concentration in each sample was brought up to 20 μ g using the vector (pCMVKM2) alone. As a control, plasmid DNA of the native Gag expression cassette was handled in the same manner. Twelve groups of four Balb/c mice (Charles River, Boston, MA) were intramuscularly immunized (50 μ l per leg,
25 intramuscular injection into the *tibialis anterior*) according to the schedule in Table 7.

Table 7

Group	Gag Expression Cassette	Concentration of Gag plasmid DNA (μ g)	Immunized at time (weeks):
1	Synthetic	20	0 ¹ , 4
2	Synthetic	2	0, 4
3	Synthetic	0.2	0, 4
4	Synthetic	0.02	0, 4
5	Synthetic	20	0
6	Synthetic	2	0
7	Synthetic	0.2	0
8	Synthetic	0.02	0
9	Native	20	0
10	Native	2	0
11	Native	0.2	0
12	Native	0.02	0

1 = initial immunization at "week 0"

Groups 1-4 were bled at week 0 (before immunization), week 4, week 6, week 8, and week 12. Groups 5-12 were bled at week 0 (before immunization) and at week 4.

B. Humoral Immune Response

The humoral immune response was checked with an anti-HIV Gag antibody ELISAs (enzyme-linked immunosorbent assays) of the mice sera 0 and 4 weeks post immunization (groups 5-12) and, in addition, 6 and 8 weeks post immunization, respectively, 2 and 4 weeks post second immunization (groups 1-4).

The antibody titers of the sera were determined by anti-Gag antibody ELISA. Briefly, sera from immunized mice were screened for antibodies directed against the HIV p55

Gag protein. ELISA microtiter plates were coated with 0.2 μ g of HIV-1_{SF2} p24-Gag protein per well overnight and washed four times; subsequently, blocking was done with PBS-0.2% Tween (Sigma) for 2 hours. After removal of the blocking solution, 100 μ l of diluted mouse serum was added. Sera were tested at 1/25 dilutions and by serial 3-fold dilutions, thereafter. Microtiter plates were washed four times and incubated with a secondary, peroxidase-coupled anti-mouse IgG antibody (Pierce, Rockford, IL). ELISA plates were washed and 100 μ l of 3, 3', 5, 5'-tetramethyl benzidine (TMB; Pierce) was added per well. The optical density of each well was measured after 15 minutes. The titers reported are the reciprocal of the dilution of serum that gave a half-maximum optical density (O.D.). The ELISA results are presented in Table 8.

Table 8

Group	Inoculum (μ g)	Expression cassette	Sera - Week 4 ³	Sera - Week 6	Sera - Week 8
1	20	S ¹ - gag	98	455	551
2	2	S - gag	59	1408	227
3	0.	S - gag	29	186	61
4	0.02	S - gag	< 20	< 20	< 20
5	20	S - gag	67	n.a. ⁴	n.a.
6	2	S - gag	63	n.a.	n.a.
7	0.	S - gag	57	n.a.	n.a.
8	0.02	S - gag	< 20	n.a.	n.a.
9	20	N ² - gag	43	n.a.	n.a.
10	2	N - gag	< 20	n.a.	n.a.
11	0.	N - gag	< 20	n.a.	n.a.
12	0.02	N - gag	< 20	n.a.	n.a.

1 = synthetic gag expression cassette (SEQ ID NO: 4)

2 = native gag expression cassette (SEQ ID NO: 1)

3 = geometric mean antibody titer

4 = not applicable

The results of the mouse immunizations with plasmid-DNAs show that the synthetic expression cassettes provide a clear improvement of immunogenicity relative to the native expression cassettes. Also, the second boost immunization
5 induced a secondary immune response after two weeks (groups 1-3).

C. Cellular Immune Response

The frequency of specific cytotoxic T-lymphocytes (CTL)
10 was evaluated by a standard chromium release assay of peptide pulsed Balb/c mouse CD4 cells. Gag expressing vaccinia virus infected CD-8 cells were used as a positive control (vvGag). Briefly, spleen cells (Effector cells, E) were obtained from the BALB/c mice immunized as described
15 above (Table 8) were cultured, restimulated, and assayed for CTL activity against Gag peptide-pulsed target cells as described (Doe, B., and Walker, C.M., *AIDS* 10(7):793-794, 1996). The HIV-1_{sf2} Gag peptide used was p7g SEQ ID NO:10. Cytotoxic activity was measured in a standard ⁵¹Cr release
20 assay. Target (T) cells were cultured with effector (E) cells at various E:T ratios for 4 hours and the average cpm from duplicate wells was used to calculate percent specific ⁵¹Cr release. The results are presented in Table 9.

Cytotoxic T-cell (CTL) activity was measured in
25 splenocytes recovered from the mice immunized with HIV Gag DNA (compare Effector column, Table 9, to immunization schedule, Table 8). Effector cells from the Gag DNA-immunized animals exhibited specific lysis of Gag p7g peptide-pulsed SV-BALB (MHC matched) targets cells
30 indicative of a CTL response. Target cells that were

peptide-pulsed and derived from an MHC-unmatched mouse strain (MC57) were not lysed (Table 9; MC/p7g).

Table 9

5

Table 9. Cytotoxic T-lymphocyte (CTL) responses in mice immunized with HIV-1 gag DNA				
Immunization	E:T	Percent specific lysis of target cells*		
		SVBALB none	SVBALB p7g	RMA p7g
20 µg DNA gagmod	100:1	2	49	<1
	30:1	3	30	<1
	10:1	<1	14	<1
2 µg DNA gagmod	100:1	2	37	<1
	30:1	2	21	<1
	10:1	<1	13	<1
0.2 µg DNA gagmod	100:1	2	32	<1
	30:1	3	25	<1
	10:1	1	14	<1
0.02 µg DNA gagmod	100:1	1	17	<1
	30:1	1	16	<1
	10:1	1	8	<1
20 µg DNA gag native	100:1	2	49	<1
	30:1	2	24	<1
	10:1	1	12	<1
2 µg DNA gag native	100:1	<1	18	<1
	30:1	1	14	<1
	10:1	1	7	<1
0.2 µg DNA gag native	100:1	3	30	<1
	30:1	3	17	<1
	10:1	2	7	<1
0.02 µg DNA gag native	100:1	4	2	<1
	30:1	1	2	<1
	10:1	1	2	<1

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*representative results of two animals per DNA-dose; positive CTL responses are indicated by boxed data

5 The results of the CTL assays show increased potency of synthetic Gag expression cassettes for induction of cytotoxic T-lymphocyte (CTL) responses by DNA immunization.

Example 9

In vivo Immunization with Env polypeptides

10 A. Immunogenicity Study of US4 o-gp140 in Ras-3c Adjuvant System

Studies have been conducted using rabbits immunized with US4 o-gp140 purified as described above. Studies are also underway in animals to determine immunogenicity of US4 gp120,
15 SF162 o-gp140 and SF162 gp120.

Two rabbits (#1 and #2) were immunized intramuscularly at 0, 4, 12 and 24 weeks with 50 μ g of US4 o-gp140 in the Ribi™ adjuvant system (RAS-3c), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more
20 bacterial cell wall components from the group consisting of monophosphorylipid A (MPL, Ribi Immunochem, Hamilton, MT). In each experiment described herein, o-gp140 can be native, mutated and/or modified. Antibody responses directed against the US4 o-gp140 protein were measured by ELISA. Results are
25 shown in Table 10.

Table 10

Rabbit/sample	Approximate o-gp140 ELISA titer
pre-immunization	0
#1: post1 (0 week immuniz)	400
#1: post2 (4 week immuniz)	15,000
#1: post3 (12 week immuniz)	50,000
#1: post4 (24 week immuniz)	100,000
#2: post1 (0 week immuniz)	600
#2: post2 (4 week immuniz)	12,000
#2: post3 (12 week immuniz)	25,000
#2: post4 (24 week immuniz)	55,000

The avidities of antibodies directed against the US4 o-gp140 protein were measured in a similar ELISA format employing successive washes with increasing concentrations of ammonium isothiocyanate. Results are shown in Table 11.

Table 11

Time of sample	Approx. Antibody avidity (NH ₄ HCN Conc. in M)
pre-immunization	0.02
post1 (0 week immuniz)	1.8
post2 (4 week immuniz)	3.5
post3 (12 week immuniz)	5.5
post4 (24 week immuniz)	5.1

These results show that US4 o-gp140 is highly immunogenic and able to induce substantial antibody responses after only one or two immunizations.

5 B. Immunogenicity of US4 o-gp140 in MF59-based Adjuvants

Groups of 4 rabbits were immunized intramuscularly at 0, 4, 12 and 24 weeks with various doses of US4 o-gp140 protein in three different MF59-based adjuvants (MF59 is described in International Publication No. WO 90/14837 and typically
10 contains 5% Squalene, 0.5% Tween 80, and 0.5% Span 85). Antibody titers were measured post-third by ELISA using SF2 gp120 to coat the plates. QHC is a quill-based adjuvant (Iscotek, Uppsala, Sweden). Results are shown in Table 12.

15 Table 12

Antigen dose (μ g)	Adjuvant	Anti-gp120 _{SF2} Ab GMT*
12.5	MF59	7231
25	MF59	8896
50	MF59	12822
12.5	MF59/MPL	24146
25	MF59/MPL	27199
50	MF59/MPL	23059
50	MF59/MPL/QHC	31759

25 *GMT = geometric mean titer

Thus, adjuvanted o-gp140 generated antigen-specific antibodies. Further, the antibodies were shown to increased in avidity over time.

C. Neutralizing Antibodies

Neutralizing antibodies post-third immunization were measured against HIV-1 SF2 in a T-cell line adapted virus (TCLA) assay and against PBMC-grown HIV-1 variants SF2, SF162
5 and 119 using the CCR5+ CEMx174 LTR-GFP reporter cell line, 5.25 (provided by N. Landau, Salk Institute, San Diego, CA) as target cells. Results are shown in Table 13.

Table 13

Neutralizing antibody responses in rabbits immunized with
o-gp140.modUS4 protein

Group	Animal	SF2 TCLA*	SF2 PBMC#	SF162 PBMC#	119 PBMC#
Experiment 1					
o-gp140/ Ras-3c 50 mg	217	>640	100%	49	17
	218	>640	96	37	29
Experiment 2					
o-gp140/ MF59 50 mg	792	45	71	39	26
	793	50	87	26	4
	794	59	87	13	0
	795	128	92	15	0
o-gp140/ MF59 + MPL 50 mg	804	173	91	47	18
	805	134	93	28	4
	806	N.D.**	95	49	13
	807	441	100	31	15
o-gp140/MF59 + MPL + QHC 50 mg	808	465	98	46	40
	809	496	100	44	39
	810	>640	101	27	4
	811	92	92	24	37

*TCLA neutralizing antibody titers (50% inhibition).

**Not Determined

% Inhibition at 1:10 dilution of sera with any detectable
non-specific inhibition in pre-bleeds subtracted.

The above studies in rabbits indicate that the US4 o-
gp140 protein is highly immunogenic. When administered with

adjuvant, this protein was able to induce substantial antibody responses after only one or two immunizations. Moreover, the adjuvanted o-gp140 protein was able to generate antigen-specific antibodies which increased in avidity after successive immunizations, and substantial neutralizing activity against T-cell line adapted HIV-1. Neutralizing activity was also observed against PBMC-grown primary HIV strains, including the difficult to neutralize CCR5 co-receptor (R5)-utilizing isolates, SF162 and 119.

10

Example 10

In Vivo Immunogenicity of Synthetic Env Expression Cassettes

A. General Immunization Methods

To evaluate the immunogenicity of the synthetic Env expression cassettes, studies using guinea pigs, rabbits, mice, rhesus macaques and baboons were performed. The studies were structured as follows: DNA immunization alone (single or multiple); DNA immunization followed by protein immunization (boost); DNA immunization followed by Sindbis particle immunization; immunization by Sindbis particles alone.

20

B. Humoral Immune Response

The humoral immune response was checked in serum specimens from immunized animals with an anti-HIV Env antibody ELISAs (enzyme-linked immunosorbent assays) at various times post-immunization. The antibody titers of the sera were determined by anti-Env antibody ELISA as described above. Briefly, sera from immunized animals were screened for antibodies directed against the HIV gp120 or gp140 Env protein. Wells of ELISA microtiter plates were coated

25

overnight with the selected *Env* protein and washed four times; subsequently, blocking was done with PBS-0.2% Tween (Sigma) for 2 hours. After removal of the blocking solution, 100 μ l of diluted mouse serum was added. Sera were tested at 1/25 dilutions and by serial 3-fold dilutions, thereafter. Microtiter plates were washed four times and incubated with a secondary, peroxidase-coupled anti-mouse IgG antibody (Pierce, Rockford, IL). ELISA plates were washed and 100 μ l of 3, 3', 5, 5'-tetramethyl benzidine (TMB; Pierce) was added per well. The optical density of each well was measured after 15 minutes. Titers are typically reported as the reciprocal of the dilution of serum that gave a half-maximum optical density (O.D.).

Example 11

DNA-immunization of Baboons Using Synthetic Gag Expression Cassettes

A. Baboons

Four baboons were immunized 3 times (weeks 0, 4 and 8) bilaterally, intramuscular into the quadriceps using 1mg pCMVKM2.GagMod.SF2 plasmid-DNA (Example 1). The animals were bled two weeks after each immunization and a p24 antibody ELISA was performed with isolated plasma. The ELISA was performed essentially as described in Example 5 except the second antibody-conjugate was an anti-human IgG, g-chain specific, peroxidase conjugate (Sigma Chemical Co., St. Louis, MD 63178) used at a dilution of 1:500. Fifty μ g/ml yeast extract was added to the dilutions of plasma samples and antibody conjugate to reduce non-specific background due to

preexisting yeast antibodies in the baboons. The antibody titer results are presented in Table 14.

Table 14

Immunization no.	Weeks	Antigen	wpi ^a / Baboon No.	Ab-titer ^b
1	0	gagmod	0 w/219	< 10
		DNA	0 w/220	< 10
			0 w/221	< 10
			0 w/222	< 10
2	6		2 wp 1st/219	< 10
			2 wp 1st/220	< 10
			2 wp 1st/221	< 10
			2 wp 1st/222	15
4	14	gagmod	2 wp 4th/219	< 10
		DNA	2 wp 4th/220	88
			2 wp 4th/221	< 10
			2 wp 4th/222	56
5	30	gagmod	2 wp 5th/219	< 10
		DNA	2 wp 5th/220	391
			2 wp 5th/221	237
			2 wp 5th/222	222
6	46	gag VLP	2 wp 6th/219	753
		protein	2 wp 6th/219	4330
			2 wp 6th/219	5000
			2 wp 6th/219	2881

^a wpi = weeks post immunization

^b geometric mean antibody titer

In Table 14, pre-bleed data are given as Immunization No. 0; data for bleeds taken 2 weeks post-first immunization are given as Immunization No. 1; data for bleeds taken 2 weeks post-second immunization are given as Immunization No. 2; and, data for bleeds taken 2 weeks post-third immunization are given as Immunization No. 3.

Further, lymphoproliferative responses to p24 antigen were also observed in baboons 221 and 222 two weeks post-fourth immunization (at week 14), and enhanced substantially post-boosting with VLP (at week 44 and 76). Such proliferation results are indicative of induction of T-helper cell functions.

B. Rhesus Macaques

The improved potency of the codon-modified *gag* expression plasmid observed in mouse and baboon studies was confirmed in rhesus macaques. Four of four macaques had detectable Gag-specific CTL after two or three 1 mg doses of modified *gag* plasmid. In contrast, in a previous study, only one of four macaques given 1 mg doses of plasmid-DNA encoding the wild-type HIV-1_{SF2} Gag showed strong CTL activity that was not apparent until after the seventh immunization. Further evidence of the potency of the modified *gag* plasmid was the observation that CTL from two of the four rhesus macaques reacted with three nonoverlapping Gag peptide pools, suggesting that as many as three different Gag peptides are recognized and indicating that the CTL response is polyclonal. Additional quantification and specificity studies are in progress to further characterize the T cell responses to Gag in the plasmid-immunized rhesus macaques. DNA immunization of macaques with the modified *gag* plasmid did not result in significant antibody responses, with only two of four animals seroconverting at low titers. In contrast, in the same study the majority of macaques in groups immunized with

p55Gag protein seroconverted and had strong Gag-specific antibody titers. These data suggest that a prime-boost strategy (DNA-prime and protein-boost) could be very promising for the induction of a strong CTL and antibody response.

In sum, these results demonstrate that the synthetic Gag plasmid DNA is immunogenic in non-human primates. When similar experiments were carried out using wild-type Gag plasmid DNA no such induction of anti-p24 antibodies was observed after four immunizations.

Example 12

DNA- and Protein Immunizations of Animals Using Env Expression Cassettes and Polypeptides

A. Guinea Pigs

Groups comprising six guinea pigs each were immunized intramuscularly at 0, 4, and 12 weeks with plasmid DNAs encoding the gp120.modUS4, gp140.modUS4, gp140.modUS4.delV1, gp140.modUS4.delV2, gp140.modUS4.delV1/V2, or gp160.modUS4 coding sequences of the US4-derived Env. The animals were subsequently boosted at 18 weeks with a single intramuscular dose of US4 o-gp140.mut.modUS4 protein in MF59 adjuvant. Anti-gp120 SF2 antibody titers (geometric mean titers) were measured at two weeks following the third DNA immunization and at two weeks after the protein boost. Results are shown in Table 15.

Table 15

Group	GMT post-DNA immuniz.	GMT post-protein boost
gp120.modUS4	2098	9489
gp140.modUS4	190	5340
gp140.modUS4.delV1	341	7808
gp140.modUS4.delV2	386	8165
gp140.modUS4.delV1/V2	664	8270
gp160.modUS4	235	9928

These results demonstrate the usefulness of the synthetic constructs to generate immune responses, as well as, the advantage of providing a protein boost to enhance the immune response following DNA immunization.

B. Rabbits

Rabbits were immunized intramuscularly and intradermally using a Bioject needleless syringe with plasmid DNAs encoding the following synthetic SF162 Env polypeptides: gp120.modSF162, gp120.modSF162.delV2, gp140.modSF162, gp140.modSF162.delV2, gp140.mut.modSF162, gp140.mut.modSF162.delV2, gp160.modSF162, and gp160.modSF162.delV2. Approximately 1 mg of plasmid DNA (pCMVlink) carrying the synthetic Env expression cassette was used to immunize the rabbits. Rabbits were immunized with plasmid DNA at 0, 4, and 12 weeks. At two weeks after the third immunization all of the constructs were shown to have generated significant antibody titers in the test animals. Further, rabbits immunized with constructs

containing deletions of the V2 region generally generated similar antibody titers relative to rabbits immunized with the companion construct still containing the V2 region.

The nucleic acid immunizations are followed by protein boosting with o-gp140.modSF162.delV2 (0.1 mg of purified protein) at 24 weeks after the initial immunization. Results are shown in Table 16.

Table 16

Group	GMT 2wks post-2nd DNA immunization	GMT 2wks post-3rd DNA immunization	GMT 2wks post-protein boost
gp120.modSF162	4573	5899	26033
gp120.modSF162.delV2	3811	3122	29606
gp140.modSF162	1478	710	12882
gp140.modSF162.delV2	1572	819	11067
gp140.mut.modSF162	1417	788	8827
gp140.mut.modSF162.delV2	1378	1207	13301
gp160.modSF162	23	81	7050
gp160.modSF162.delV2	85	459	11568

All constructs are highly immunogenic and generate substantial antigen binding antibody responses after only 2 immunizations in rabbits.

C. Baboons

Groups of four baboons were immunized intramuscularly with 1 mg doses of DNA encoding different forms of synthetic US4 gp140 (see the following table) at 0, 4, 8, 12, 28, and 44 weeks. The animals were also boosted twice with US4 0-

1621.002
2302-1621
PATENT

gp140 protein (gp140.mut.modUS4) at 44 and 76 weeks using
MF59 as adjuvant. Results are shown in Table 17.

Table 17				
Animal	Treatment	2 Wks Post 5th DNA immuniza- tion	2 Wks post 6th DNA (plus o- gp140 prot. immuniz.)	2 Wks post 7th DNA (o- gp140 protein only)
CY 215	gp140.modUS4	8.3	446	1813
CY 216		8.3	433	1236
CY 217		68	1660	2989
CY 218		101	2556	1610
Geomean:		26.2	951.4	1812.1
CY 219	gp140.modUS4 + p55gag.SF2	8.3	8.3	421
CY 220		8.3	8.3	3117
CY 221		8.3	954	871
CY 222		8.3	71	916
Geomean:		8.3	46.5	1011.5
CY 223	gp140.mut. modUS4	41.4	10497	46432
CY 224		8.3	979	470
CY 225		135	2935	3870
CY 226		47	1209	4009
Geomean:		68.3	2457.4	4289.6
CY 227	gp140TM. modUS4	8.3	56	5001
CY 228		8.3	806	1170
CY 229		8.3	48	3402
CY 230		8.3	38	6520
GMT*:		8.3	95.3	3375.3

*GMT = geometric mean titer

The results in Table 17 demonstrate the usefulness of
the synthetic constructs to generate immune responses in

primates such as baboons. In addition, all animals showed evidence of antigen-specific (*Env* antigen) lymphoproliferative responses.

5 D. Rhesus Macaques

Two rhesus macaques (designated H445 and J408) were immunized with 1 mg of DNA encoding SF162 gp140 with a deleted V2 region (SF162.gp140.delV2) by intramuscular (IM) and intradermal (ID) routes at 0, 4, 8, and 28 weeks.

10 Approximately 100 μ g of the protein encoded by the SF162.gp140mut.delV2 construct was also administered in MF59 by IM delivery at 28 weeks.

ELISA titers are shown in Figure 61. Neutralizing antibody activity is shown Tables 18 and 19. Neutralizing
15 antibody activity was determined against a variety of primary HIV-1 isolates in a primary lymphocyte or "PBMC-based" assay (see the following tables). Further, the phenotypic co-receptor usage for each of the primary isolates is indicated. As can be seen in the tables
20 neutralizing antibodies were detected against every isolate tested, including the HIV-1 primary isolates (i.e., SF128A, 92US660, 92HT593, 92US657, 92US714, 91US056, and 91US054).

Table 18					
	Treatment		Bleed 0	Bleed 1	Bleed 2
Animal	1st Immunization	2nd Immunization	1st Imm'n	2nd Imm'n	2 Wks post 2nd
5	EO 456	(None)	8.3	45	309
	EO 457		8.3	254	460
	EO 458		8.3	8.3	93
	EO 459		8.3	43	45
	EO 460		8.3	8.3	274
10	EO 461	25µg 120mod DNA	8.3	47	1502
	EO 462		8.3	80	5776
	EO 463		8.3	89	3440
	EO 464		8.3	8.3	3347
	EO 465		8.3	69	1127
15	EO 466	(None)	8.3	63	102
	EO 467		8.3	112	662
	EO 468		8.3	94	459
	EO 469		8.3	58	48
	EO 470		8.3	95	355
20	EO 471	50µg 120mod DNA	8.3	110	9074
	EO 472		8.3	8.3	4897
	EO 473		8.3	49	4089
	EO 474		8.3	59	5280
	EO 475		8.3	8.3	929
25	EO 476	25µg 120mod DNA	8.3		653
	EO 477		8.3	87	22675
	EO 478		8.3	76	3869
	EO 479		8.3		1004
	EO 480		8.3	71	7080

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Table 19					
	Treatment		Bleed 0	Bleed 1	Bleed 2
Animal	1st Immunization	2nd Immunization	1st Imm'n	2nd Imm'n	2 Wks post 2nd
EO 481	Sindbis/Env	(None)	8.3	8.3	8.3
EO 482			8.3	8.3	8.3
EO 483			8.3	78	103
EO 484			8.3	8.3	32
EO 485			8.3	76	207
EO 486	Sindbis/Env	Sindbis/Env	8.3	8.3	458
EO 487			8.3	8.3	345
EO 488			8.3	8.3	331
EO 489			8.3	103	111
EO 490			8.3	8.3	5636

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Lymphoproliferative activity (LPA) was also determined by antigenic stimulation followed by uptake of ³H-thymidine in these animals and is shown in Table 20. Experiment 1 was performed at 14 weeks post third DNA immunization and Experiment 2 was performed at 2 weeks post fourth DNA immunization using DNA and protein. For gp120ThaiE, gp120SF2 and US4 o-gp140, appropriate background values were used to calculate Stimulation Indices (S.I.; Antigenic stimulation CPM/Background CPM).

Table 20

S.I.: Calculated as Ag CPM/Background CPM				
Animal/ exp#	gp120ThaiE	gp120 SF2	env2-3SF2	o-gp140US4
J408/#1	2	1	1	5
H445/#1	1	1	1	6
J408/#2	1	1	2	3
H445/#2	0	0	3	2

As can be seen by the results presented in Table 20 lymphoproliferative responses to o-gp140.US4 antigen were also in all four animals at both experimental time points. Such proliferation results are indicative of induction of T-helper cell functions.

The results presented above demonstrate that the synthetic gp140.modSF162.delV2 DNA and protein are immunogenic in non-human primates.

Example 13

In vitro expression of recombinant Sindbis RNA and DNA
containing the synthetic Gag or Env expression cassettes

A. Synthetic Gag expression cassettes

5 To evaluate the expression efficiency of the synthetic
Gag expression cassette in Alphavirus vectors, the synthetic
Gag expression cassette was subcloned into both plasmid DNA-
based and recombinant vector particle-based Sindbis virus
vectors. Specifically, a cDNA vector construct for *in vitro*
10 transcription of Sindbis virus RNA vector replicons (pRSIN-
luc; Dubensky, et al., *J Virol.* 70:508-519, 1996) was
modified to contain a *PmeI* site for plasmid linearization
and a polylinker for insertion of heterologous genes. A
polylinker was generated using two oligonucleotides that
15 contain the sites *XhoI*, *PmlI*, *ApaI*, *NarI*, *XbaI*, and *NotI*
(XPANXNF, SEQ ID NO:17, and XPANXNR, SEQ ID NO:18).

The plasmid pRSIN-luc (Dubensky et al., *supra*) was
digested with *XhoI* and *NotI* to remove the luciferase gene
insert, blunt-ended using Klenow and dNTPs, and purified
20 from an agarose gel using GeneCleanII (Biol01, Vista, CA).
The oligonucleotides were annealed to each other and ligated
into the plasmid. The resulting construct was digested with
NotI and *SacI* to remove the minimal Sindbis 3'-end sequence
and A₄₀ tract, and ligated with an approximately 0.4 kbp
25 fragment from PKSSIN1-BV (WO 97/38087). This 0.4 kbp
fragment was obtained by digestion of pKSSIN1-BV with *NotI*
and *SacI*, and purification after size fractionation from an
agarose gel. The fragment contained the complete Sindbis
virus 3'-end, an A₄₀ tract and a *PmeI* site for

linearization. This new vector construct was designated SINBVE.

The synthetic HIV Gag coding sequence was obtained from the parental plasmid by digestion with *EcoRI*, blunt-ending with Klenow and dNTPs, purification with GeneCleanII, digestion with *SalI*, size fractionation on an agarose gel, and purification from the agarose gel using GeneCleanII.

The synthetic Gag coding fragment was ligated into the SINBVE vector that had been digested with *XhoI* and *PmlI*.

The resulting vector was purified using GeneCleanII and

designated SINBVGag. Vector RNA replicons may be transcribed *in vitro* (Dubensky et al., *supra*) from SINBVGag and used directly for transfection of cells. Alternatively, the replicons may be packaged into recombinant vector particles by co-transfection with defective helper RNAs or using an alphavirus packaging cell line as described, for example, in U.S. Patent Numbers 5,843,723 and 5,789,245, and then administered *in vivo* as described..

The DNA-based Sindbis virus vector pDCMVSIN-beta-gal (Dubensky, et al., *J Virol.* 70:508-519, 1996) was digested with *SalI* and *XbaI*, to remove the beta-galactosidase gene insert, and purified using GeneCleanII after agarose gel size fractionation. The HIV Gag gene was inserted into the the pDCMVSIN-beta-gal by digestion of SINBVGag with *SalI* and *XhoI*, purification using GeneCleanII of the Gag-containing fragment after agarose gel size fractionation, and ligation. The resulting construct was designated pDSIN-Gag, and may be used directly for *in vivo* administration or formulated using any of the methods described herein.

BHK and 293 cells were transfected with recombinant Sindbis vector RNA and DNA, respectively. The supernatants

and cell lysates were tested with the Coulter p24 capture ELISA (Example 2).

BHK cells were transfected by electroporation with recombinant Sindbis RNA. The expression of p24 (in ng/ml) is presented in Table 21. In the table, SINGag#1 and 2 represent duplicate measurements, and SIN β gal represents a negative control. Supernatants and lysates were collected 24h post transfection.

Table 21

Construct	Supernatant	Lysate
SIN β gal RNA	0	0
SINGag#1 RNA	7 ng	Max (approx. 1 μ g)
SINGag#2 RNA	1 ng	700 ng

293 cells were transfected using LT-1 (Example 2) with recombinant Sindbis DNA. Synthetic pCMVKM2GagMod.SF2 was used as a positive control. Supernatants and lysates were collected 48h post transfection. The expression of p24 (in ng/ml) is presented in Table 22.

Table 22

Construct	Supernatant	Lysate
SINGag DNA	3	30
pCMVKM2.GagMod.SF2 DNA	32	42

The results presented in Tables 21 and 22 demonstrate that Gag proteins can be efficiently expressed from both DNA and RNA-based Sindbis vector systems using the synthetic Gag expression cassette (p55Gag.mod).

B. Synthetic Env expression cassettes

To evaluate the expression efficiency of the synthetic Env expression cassette in Alphavirus vectors, synthetic Env expression cassettes were subcloned into both plasmid DNA-
5 based and recombinant vector particle-based Sindbis virus vectors as described above for Gag.

The synthetic HIV Env coding sequence was obtained from the parental plasmid by digestion with *SalI* and *XbaI*, size fractionation on an agarose gel, and purification from the
10 agarose gel using GeneCleanII. The synthetic Env coding fragment was ligated into the SINBVE vector that had been digested with *XhoI* and *XbaI*. The resulting vector was purified using GeneCleanII and designated SINBVEnv. Vector RNA replicons may be transcribed *in vitro* (Dubensky et al.,
15 *supra*) from SINBVEnv and used directly for transfection of cells. Alternatively, the replicons may be packaged into recombinant vector particles by co-transfection with defective helper RNAs or using an alphavirus packaging cell line and administered as described above for Gag.

The DNA-based Sindbis virus vector pDCMVSIN-beta-gal
20 (Dubensky, et al., *J Virol.* 70:508-519, 1996) was digested with *SalI* and *XbaI*, to remove the beta-galactosidase gene insert, and purified using GeneCleanII after agarose gel size fractionation. The HIV Env gene was inserted into the
25 the pDCMVSIN-beta-gal by digestion of SINBVEnv with *XbaI* and *XhoI*, purification using GeneCleanII of the Env-containing fragment after agarose gel size fractionation, and ligation. The resulting construct was designated pDSIN-Env, and may be used directly for *in vivo* administration or formulated using
30 any of the methods described herein.

BHK and 293 cells were transfected with recombinant Sindbis vector RNA and DNA, respectively. The supernatants and cell lysates were tested by capture ELISA.

5 BHK cells were transfected by electroporation with recombinant Sindbis RNA. The expression of Env (in ng/ml) is presented in Table 23. In the table, the Sindbis RNA containing synthetic Env expression cassettes are indicated and β gal represents a negative control. Supernatants and lysates were collected 24h post transfection.

10

Table 23

Construct	Supernatant (Neat)ng/ml	Lysate (1:10 dilution)ng/ml
β gal RNA	0	0
gp140.modUS4	726	7147
15 gp140.modSF162	3529	7772
gp140.modUS4.delV1/V2	1738	6526
gp140.modUS4.delV2	960	3023
gp140.modSF162.delV2	2772	3359

20

293 cells were transfected using LT-1 mediated transfection (PanVera) with recombinant Sindbis DNA containing synthetic expression cassettes of the present invention and β gal sequences as a negative control.

25 Supernatants and lysates were collected 48h post transfection. The expression of Env (in ng/ml) is presented in Table 24.

Table 24

Construct	Supernatant (Neat)ng/ml	Lysate (1:10 dilution)ng/ml
βgal	0	0
gp140.modSF162.delV2	1977	801
gp140.modSF162	949	746

The results presented in Tables 23 and 24 demonstrated that Env proteins can be efficiently expressed from both DNA and RNA-based Sindbis vector systems using the synthetic Env expression cassettes of the present invention.

Example 14

A. In vivo Immunization with Gag-containing DNA and/or Sindbis particles

CB6F1 mice were immunized intramuscularly at 0 and 4 weeks with plasmid DNA and/or Sindbis vector RNA-containing particles each containing GagMod.SF2 sequences as indicated in Table 25. Animals were challenged with recombinant vaccinia expressing SF2 Gag at 3 weeks post second immunization (at week 7). Spleens were removed from the immunized and challenged animals 5 days later for a standard ⁵¹C release assay for CTL activity. Values shown in Table 25 indicate the results from the spleens of three mice from each group. The boxed values in Table 25 indicate that all groups of mice receiving immunizations with pCMVKm2.GagMod.SF2 DNA and/or SindbisGagMod.SF2 virus particles either alone or in combinations showed antigen-specific CTL activity.

Table 25

Cytotoxic T-lymphocyte (CTL) responses in mice immunized with HIV-1 gagmod DNA and Sindbis gagmod virus particles				
Immunization	E:T	Percent specific lysis of target cells*		
		SVBALB none	SVBALB p7g	RMA p7g
pCMVKm2.GagMod.SF2 DNA ^a	100:1	5	20	1
at 0, 4 wks	25:1	5	20	<1
	6:1	4	8	<1
SindbisGagMod.SF2 virus particles ^b	100:1	10	49	<1
at 0, 4 weeks	25:1	7	20	<1
	6:1	5	12	<1
pCMVKm2.GagMod.SF2 DNA at 0 wks	100:1	9	58	<1
SindbisGagMod.SF2 virus particles at 4 wks	25:1	7	42	2
	6:1	4	13	<1
SindbisGagMod.SF2 virus particles at 4 wks	100:1	5	38	<1
	25:1	4	18	<1
pCMVKm2.GagMod.SF2 DNA at 0 wks	6:1	3	13	1

^a 20 µg

^b 10⁷ particles

* Challenge with recombinant vaccinia virus expressing HIV-1SF2 Gag at 3 weeks post second immunization (week 7). Spleens taken 5 days later. Ex vivo CTL assay performed by standard ⁵¹Cr release assay. Values seen represent results from 3 pooled mouse spleens per group

B. In vivo Immunization with Env-containing DNA and/or Sindbis particles

Balb/C mice were immunized intramuscularly at 0 and 4 weeks(as shown in the following table) with plasmid DNA and/or Sindbis-virus RNA-containing particles each containing gp120.modUS4 sequences. Treatment regimes and antibody titers are shown in Table 26. Antibody titers were determined by ELISA using gp120 SF2 protein to coat the plates.

Table 26					
	Treatment		Bleed 0	Bleed 1 (8 wks)	Bleed 2 (10 wks)
Animal	1st Immunization	2nd Immunization	1st Imm'n	2nd Imm'n	2 Wks post 2nd
EO 456	25 μ g 120mod DNA	(None)	8.3	45	309
EO 457			8.3	254	460
EO 458			8.3	8.3	93
EO 459			8.3	43	45
EO 460			8.3	8.3	274
EO 461	25 μ g 120mod DNA	25 μ g 120mod DNA	8.3	47	1502
EO 462			8.3	80	5776
EO 463			8.3	89	3440
EO 464			8.3	8.3	3347
EO 465			8.3	69	1127
EO 466	50 μ g 120mod DNA	(None)	8.3	63	102
EO 467			8.3	112	662
EO 468			8.3	94	459
EO 469			8.3	58	48
EO 470			8.3	95	355
EO 471	50 μ g 120mod DNA	50 μ g 120mod DNA	8.3	110	9074
EO 472			8.3	8.3	4897
EO 473			8.3	49	4089
EO 474			8.3	59	5280
EO 475			8.3	8.3	929
EO 476	25 μ g 120mod DNA	Sindbis/Env	8.3		653
EO 477			8.3	87	22675
EO 478			8.3	76	3869
EO 479			8.3		1004
EO 480			8.3	71	7080
EO 481	Sindbis/Env	(None)	8.3	8.3	8.3
EO 482			8.3	8.3	8.3
EO 483			8.3	78	103
EO 484			8.3	8.3	32
EO 485			8.3	76	207
EO 486	Sindbis/Env	Sindbis/Env	8.3	8.3	458
EO 487			8.3	8.3	345
EO 488			8.3	8.3	331
EO 489			8.3	103	111
EO 490			8.3	8.3	5636

As can be seen from the data presented above, all of the mice generally demonstrated substantial immunological

responses by bleed number 2. For Env, the best results were obtained using either (i) 50 μ g of gp120.modUS4 DNA for the first immunization followed by a second immunization using 50 μ g of gp120.modUS4 DNA, or (ii) 25 μ g of gp120.modUS4 DNA for the first immunization followed by a second immunization using 10^7 pfus of Sindbis.

The results presented above demonstrate that the Env and Gag proteins of the present invention are effective to induce an immune response using Sindbis vector systems which include the synthetic Env (e.g., gp120.modUS4) or Gag expression cassettes.

Example 15

Co-Transfection of Env and Gag as Monocistronic and Bicistronic Constructs

DNA constructs encoding (i) wild-type US4 and SF162 Env polypeptides, (ii) synthetic US4 and SF162 Env polypeptides (gp160.modUS4, gp160.modUS4.delV1/V2, gp160.modSF162, and gp120.modSF162.delV2), and (iii) SF2gag polypeptide (i.e., the Gag coding sequences obtained from the SF2 variant or optimized sequences corresponding to the gagSF2 -- gag.modSF2) were prepared. These monocistronic constructs were co-transfected into 293T cells in a transient transfection protocol using the following combinations:

gp160.modUS4; gp160.modUS4 and gag.modSF2;
gp160.modUS4.delV1/V2; gp160.modUS4.delV1/V2 and gag.modSF2;
gp160.modSF162 and gag.modSF2; gp120.modSF162.delV2 and gag.modSF2; and gag.modSF2 alone.

Further several bicistronic constructs were made where the coding sequences for Env and Gag were under the control of a single CMV promoter and, between the two coding

sequences, an IRES (internal ribosome entry site (EMCV IRES); Kozak, M., Critical Reviews in Biochemistry and Molecular Biology 27(45):385-402, 1992; Witherell, G.W., et al., Virology 214:660-663, 1995) sequence was introduced
5 after the Env coding sequence and before the Gag coding sequence. Those constructs were as follows:
gp160.modUS4.gag.modSF2, SEQ ID NO:73 (Figure 61);
gp160.modUSF162.gag.modSF2, SEQ ID NO:74 (Figure 62);
gp160.modUS4.delV1/V2.gag.modSF2, SEQ ID NO:75 (Figure 63);
10 and gp160.modSF162.delV2.gag.modSF2, SEQ ID NO:76 (Figure 64).

Supernatants from cell culture were filtered through 0.45 μ m filters then ultracentrifuged for 2 hours at 24,000 rpm (140,000Xg) in an SW28 rotor through a 20% sucrose
15 cushion. The pelleted materials were suspended and layered on a 20-60% sucrose gradient and spun for 2 hours at 40,000 rpm (285,000Xg) in an SW41Ti rotor. Gradients were fractionated into 1.0 ml samples. A total of 9-10 fractions were typically collected from each DNA transfection group.

20 The fractions were tested for the presence of the Env and Gag proteins (across all fractions). These results demonstrated that the appropriate proteins were expressed in the transfected cells (i.e., if an Env coding sequence was present the corresponding Env protein was detected; if a Gag
25 coding sequence was present the corresponding Gag protein was detected).

Virus like particles (VLPs) were known to be present through a selected range of sucrose densities. Chimeric virus like particles (VLPs) were formed using all the tested
30 combinations of constructs containing both Env and Gag. Significantly more protein was found in the supernatant collected from the cells transfected with

"gp160.modUS4.delV1/V2 and gag.modSF2" than in all the other supernatants.

Western blot analysis was also performed on sucrose gradient fractions from each transfection. The results show
5 that bicistronic plasmids gave lower amounts of VLPs than the amounts obtained using co-transfection with monocistronic plasmids.

In order to verify the production of chimeric VLPs by these cell lines the following electron microscopic analysis
10 was carried out.

293T cells were plated at a density of 60-70% confluence in 100 mm dishes on the day before transfection. The cells were transfected with 10 μ g of DNA in transfection reagent LT1 (Panvera Corporation, 545 Science Dr., Madison,
15 WI). The cells were incubated overnight in reduced serum medium (opti-MEM, Gibco-BRL, Gaithersburg, MD). The medium was replaced with 10% fetal calf serum, 2% glutamine in IMDM in the morning of the next day and the cells were incubated for 65 hours. Supernatants and lysates were collected for
20 analysis as described above (see Example 2).

The fixed, transfected 293T cells and purified ENV-GAG VLPs were analyzed by electron microscopy. The cells were fixed as follows. Cell monolayers were washed twice with PBS and fixed with 2% glutaraldehyde. For purified VLPs,
25 gradient peak fractions were collected and concentrated by ultracentrifugation (24,000 rpm) for 2 hours. Electron microscopic analysis was performed by Prof. T.S. Benedict Yen (Veterans Affairs, Medical Center, San Francisco, CA).

Electron microscopy was carried out using a
30 transmission electron microscope (Zeiss 10c). The cells were pre-stained with osmium and stained with uranium acetate and lead citrate. Immunostaining was performed to

visualize envelope on the VLP. The magnification was 100,000X.

5 Figures 65A-65F show micrographs of 293T cells transfected with the following constructs: Figure 65A, gag.modSF2; Figure 65B, gp160.modUS4; Figure 65C, gp160.modUS4.delV1/V2.gag.modSF2 (bicistronic Env and Gag); Figures 65D and 65E, gp160.modUS4.delV1/V2 and gag.modSF2; and Figure 65F, gp120.modSF162.delV2 and gag.modSF2. In the figures, free and budding immature virus-like-particles (VLPs) of the expected size (approximately 100 nm) decorated with the Env protein were seen. In sum, gp160 polypeptides incorporate into Gag VLPs when constructs were co-transfected into cells. The efficiency of incorporation is 2-3 fold higher when constructs encoding V-deleted Env polypeptides from high synthetic expression cassettes are used.

10
15

Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

20

What Is Claimed Is:

1. An expression cassette, comprising
5 a polynucleotide sequence encoding a polypeptide including an HIV Gag polypeptide, wherein the polynucleotide sequence encoding said Gag polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:20.

10

2. The expression cassette of claim 1, comprising, a polynucleotide sequence encoding a polypeptide including an HIV Gag polypeptide, wherein the polynucleotide sequence encoding said Gag polypeptide comprises a sequence
15 having at least 90% sequence identity to the sequence presented as SEQ ID NO:9.

3. The expression cassette of claim 1, wherein said polynucleotide sequence encoding a polypeptide including an
20 HIV Gag polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:4.

4. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide
25 sequence encoding an HIV *protease* polypeptide.

5. The expression cassette of claim 4, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to a sequence
30 selected from the group consisting of: SEQ ID NO:5, SEQ ID NO:78, and SEQ ID NO:79.

6. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV *reverse transcriptase* polypeptide.

5 7. The expression cassette of claim 6, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, and SEQ ID NO:84.

10 8. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV *tat* polypeptide.

15 9. The expression cassette of claim 8, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to a sequence selected from the group consisting of: SEQ ID NO:87, SEQ ID NO:88, and SEQ ID NO:89.

20 10. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV *polymerase* polypeptide, wherein the nucleotide sequence encoding said polypeptide comprises a
25 sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:6.

30 11. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV *polymerase* polypeptide, wherein (i) the nucleotide sequence encoding said polypeptide comprises

a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:4, and (ii) wherein the sequence is modified by deletions of coding regions corresponding to reverse transcriptase and integrase.

5

12. The expression cassette of claim 11, wherein said polynucleotide sequence preserves T-helper cell and CTL epitopes.

10

13. The expression cassette of claim 1, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HCV core polypeptide, wherein the nucleotide sequence encoding said polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as SEQ ID NO:7.

15

14. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to SEQ ID NO:71 (Figure 58) or SEQ ID NO:72 (Figure 59).

20

15. The expression cassette of claim 14, wherein said *Env* polypeptide includes sequences flanking a V1 region but has a deletion in the V1 region itself.

25

16. The expression cassette of claim 15, wherein the polynucleotide sequence encoding the polypeptide comprises the sequence presented as SEQ ID NO:65 (Figure 52 gp160.modUS4.delV1).

30

17. The expression cassette of claim 14, wherein said *Env* polypeptide includes sequences flanking a V2 region but has a deletion in the V2 region itself.

5 18. The expression cassette of claim 17, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:60 (Figure 47); and SEQ ID NO:66 (Figure 53).

10 19. The expression cassette of claim 17, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:34 (Figure 20); SEQ ID NO:37 (Figure 24); SEQ ID NO:40 (Figure 27); SEQ ID NO:43 (Figure 30); SEQ ID NO:46 (Figure 33); SEQ ID NO:76 (Figure
15 64) and SEQ ID NO:49 (Figure 36).

20 20. The expression cassette of claim 14, wherein said *Env* polypeptide includes sequences flanking a V1/V2 region but has a deletion in the V1/V2 region itself.

21. The expression cassette of claim 20, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:59 (Figure 46); SEQ ID NO:61 (Figure 48); SEQ ID NO:67 (Figure 54); and SEQ ID
25 NO:75 (Figure 63).

22. The expression cassette of claim 20, wherein the polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:35 (Figure 21); SEQ
30 ID NO:38 (Figure 25); SEQ ID NO:41 (Figure 28); SEQ ID NO:44

(Figure 31); SEQ ID NO:47 (Figure 34) and SEQ ID NO:50 (Figure 37).

23. The expression cassette of claim 14, wherein said
5 *Env* polypeptide has a mutated cleavage site that prevents the cleavage of a gp140 polypeptide into a gp120 polypeptide and a gp41 polypeptide.

24. The expression cassette of claim 23, wherein the
10 polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:57 (Figure 44); SEQ ID NO:61 (Figure 48); and SEQ ID NO:63 (Figure 50).

25. The expression cassette of claim 23, wherein the
15 polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:39 (Figure 26); SEQ ID NO:40 (Figure 27); SEQ ID NO:41 (Figure 28); SEQ ID NO:42 (Figure 29); SEQ ID NO:43 (Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45 (Figure 32); SEQ ID NO:46 (Figure 33); and
20 SEQ ID NO:47 (Figure 34).

26. The expression cassette of claim 14, wherein said
Env polypeptide includes a gp160 *Env* polypeptide or a polypeptide derived from a gp160 *Env* polypeptide.

25

27. The expression cassette of claim 26, wherein the
polynucleotide sequence encoding the polypeptide is selected from the group consisting of: SEQ ID NO:64 (Figure 51); SEQ ID NO:65 (Figure 52); SEQ ID NO:66 (Figure 53); SEQ ID NO:67
30 (Figure 54); SEQ ID NO:68 (Figure 55); SEQ ID NO:75 (Figure

63); and SEQ ID NO:73 (Figure 61).

28. The expression cassette of claim 26, wherein the polynucleotide sequence encoding the polypeptide is selected
5 from the group consisting of: SEQ ID NO:48 (Figure 35); SEQ ID NO:49 (Figure 36); SEQ ID NO:50 (Figure 37); SEQ ID NO:76 (Figure 64); and SEQ ID NO:74 (Figure 62).

29. The expression cassette of claim 14, wherein said
10 *Env* polypeptide includes a gp140 *Env* polypeptide or a polypeptide derived from a gp140 *Env* polypeptide.

30. The expression cassette of claim 29, wherein the polynucleotide sequence encoding the polypeptide is selected
15 from the group consisting of: SEQ ID NO:56 (Figure 43); SEQ ID NO:57 (Figure 44); SEQ ID NO:58 (Figure 45); SEQ ID NO:59 (Figure 46); SEQ ID NO:60 (Figure 47); SEQ ID NO:61 (Figure 48); SEQ ID NO:62 (Figure 49); and SEQ ID NO:63 (Figure 50).

31. The expression cassette of claim 29, wherein the polynucleotide sequence encoding the polypeptide is selected
20 from the group consisting of: SEQ ID NO:36 (Figure 23); SEQ ID NO:37 (Figure 24); SEQ ID NO:38 (Figure 25); SEQ ID NO:39 (Figure 26); SEQ ID NO:40 (Figure 27); SEQ ID NO:41 (Figure 28); SEQ ID NO:42 (Figure 29); SEQ ID NO:43 (Figure 30); SEQ ID NO:44 (Figure 31); SEQ ID NO:45 (Figure 32); SEQ ID NO:46 (Figure 33); and SEQ ID NO:47 (Figure 34).

32. The expression cassette of claim 14, wherein said
30 *Env* polypeptide includes a gp120 *Env* polypeptide or a

polypeptide derived from a gp120 *Env* polypeptide.

33. The expression cassette of claim 32, wherein the polynucleotide sequence encoding the polypeptide is selected
5 from the group consisting of: SEQ ID NO:54 (Figure 41); and
SEQ ID NO:55 (Figure 42).

34. The expression cassette of claim 32, wherein the polynucleotide sequence encoding the polypeptide is selected
10 from the group consisting of: SEQ ID NO:33 (Figure 19); SEQ
ID NO:34 (Figure 20); and SEQ ID NO:35 (Figure 21).

35. The expression cassette of claim 14, wherein the polynucleotide sequence encoding the polypeptide is selected
15 from the group consisting of: SEQ ID NO:55 (Figure 42); SEQ
ID NO:62 (Figure 49); SEQ ID NO:63 (Figure 50); and SEQ ID
NO:68 (Figure 55).

36. A recombinant expression system for use in a
20 selected host cell, comprising, an expression cassette of
claim 1, and wherein said polynucleotide sequence is
operably linked to control elements compatible with
expression in the selected host cell.

25 37. The recombinant expression system of claim 36,
wherein said control elements are selected from the group
consisting of a transcription promoter, a transcription
enhancer element, a transcription termination signal,
polyadenylation sequences, sequences for optimization of
30 initiation of translation, and translation termination
sequences.

38. The recombinant expression system of claim 36, wherein said transcription promoter is selected from the group consisting of CMV, CMV+intron A, SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein.

5

39. A cell comprising an expression cassette of claim 1, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected cell.

10

40. The cell of claim 39, wherein the cell is a mammalian cell.

15

41. The cell of claim 40, wherein the cell is selected from the group consisting of BHK, VERO, HT1080, 293, RD, COS-7, and CHO cells.

20

42. The cell of claim 41, wherein said cell is a CHO cell.

43. The cell of claim 39, wherein the cell is an insect cell.

25

44. The cell of claim 43, wherein the cell is either *Trichoplusia ni* (Tn5) or Sf9 insect cells.

45. The cell of claim 39, wherein the cell is a bacterial cell.

30

46. The cell of claim 39, wherein the cell is a yeast cell.

47. The cell of claim 39, wherein the cell is a plant cell.

5 48. The cell of claim 39, wherein the cell is an antigen presenting cell.

10 49. The cell of claim 48, wherein the lymphoid cell is selected from the group consisting of macrophage, monocytes, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof.

15 50. The cell of claim 39, wherein the cell is a primary cell.

 51. The cell of claim 39, wherein the cell is an immortalized cell.

20 52. The cell of claim 39, wherein the cell is a tumor-derived cell.

25 53. A method for producing a polypeptide including HIV Gag polypeptide sequences, said method comprising, incubating the cells of claim 39, under conditions for producing said polypeptide.

30 54. A method for producing virus-like particles (VLPs), comprising, incubating the cells of claim 39, under conditions for producing said VLPs.

55. A method for producing a composition of virus-like particles (VLPs), comprising,

(a) incubating the cells of claim 39, under conditions for producing said VLPs; and

5 (b) substantially purifying said VLPs to produce a composition of VLPs.

56. A cell line useful for packaging lentivirus vectors, comprising

10 suitable host cells that have been transfected with an expression vector containing an expression cassette of claim 1, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell.

15 57. A cell line useful for packaging lentivirus vectors, comprising

20 suitable host cells that have been transfected with an expression vector containing an expression cassette of claim 2, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell.

25 58. A cell line useful for packaging lentivirus vectors, comprising

30 suitable host cells that have been transfected with an expression vector containing an expression cassette of claim 3, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the host cell.

59. A cell line useful for packaging lentivirus

suitable host cells that have been transfected with an expression vector containing an expression cassette of claim 11, and wherein said polynucleotide sequence is operably
5 linked to control elements compatible with expression in the host cell.

15

61. A method of DNA immunization of a subject,
comprising,
introducing a gene delivery vector of claim 60 into
said subject under conditions that are compatible with
20 expression of said expression cassette in said subject.

25 63. The method of claim 61, wherein said vector is
delivered using a particulate carrier.

65. The method of claim 63, wherein said vector is

encapsulated in a liposome preparation.

66. The method of claim 61, wherein said vector is a viral vector.

5

67. The method of claim 66, wherein said viral vector is a retroviral vector.

68. The method of claim 67, wherein said viral vector is a lentiviral vector.

10

69. The method of claim 61, wherein said subject is a mammal.

70. The method of claim 69, wherein said mammal is a human.

15

71. A method of generating an immune response in a subject, comprising

transfecting cells of said subject a gene delivery vector of claim 60, under conditions that permit the expression of said polynucleotide and production of said polypeptide, thereby eliciting an immunological response to said polypeptide.

20

25

72. The method of claim 71, wherein said vector is a nonviral vector.

73. The method of claim 72, wherein said vector is delivered using a particulate carrier.

30

74. The method of claim 73, wherein said vector is

coated on a gold or tungsten particle and said coated particle is delivered to said vertebrate cell using a gene gun.

5 75. The method of claim 73, wherein said vector is encapsulated in a liposome preparation.

76. The method of claim 71, wherein said vector is a viral vector.

10

77. The method of claim 76, wherein said viral vector is a retroviral vector.

15

78. The method of claim 77, wherein said viral vector is a lentiviral vector.

79. The method of claim 71, wherein said subject is a mammal.

20

80. The method of claim 79, wherein said mammal is a human.

25

81. The method of claim 71, wherein said transfecting is done *ex vivo* and said transfected cells are reintroduced into said subject.

82. The method of claim 71, wherein said transfecting is done *in vivo* in said subject.

30

83. The method of claim 71, where said immune response is a humoral immune response.

84. The method of claim 71, where said immune response is a cellular immune response.

5 85. A gene delivery vector comprising an alphavirus vector construct, wherein said alphavirus construct comprises an expression cassette according to claim 1.

10 86. The gene delivery vector of claim 85, wherein the alphavirus vector construct is a cDNA vector construct.

15 87. The gene delivery vector of claim 85, wherein the alphavirus comprises a recombinant alphavirus particle preparation.

 88. The gene delivery vector of claim 85, wherein the vector comprises a eukaryotic layered vector initiation system.

20 89. A method of stimulating an immune response in a subject comprising administering the gene delivery vector of claim 85 in an amount effective to stimulate an immune response in said subject.

25 90. The method of claim 89, wherein the gene delivery vector is administered intramuscularly, intramucosally, intranasally, subcutaneously, intradermally, transdermall, intravaginally, intrarectally, orally or intravenously.

30

IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND
5 PRODUCTION OF VIRUS-LIKE PARTICLES

Abstract of the Disclosure

660221-342450

10 The present invention relates to the efficient
expression of HIV polypeptides in a variety of cell types,
including, but not limited to, mammalian, insect, and plant
cells. Synthetic expression cassettes encoding the HIV Gag-
containing polypeptides are described, as are uses of the
expression cassettes in applications including DNA
15 immunization, generation of packaging cell lines, and
production of Env-, tat- or Gag-containing proteins. The
invention provides methods of producing Virus-Like Particles
(VLPs), as well as, uses of the VLPs including, but not
limited to, vehicles for the presentation of antigens and
20 stimulation of immune response in subjects to whom the VLPs
are administered.

orig.gagSF2

ATGGGTGCGAGAGCGTCGGTATTAAGCGGGGAGAATTAGATAAAATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAG

AAAAATATAAGTTAAACATATAGTATGGGCAAGCAGGGAGCTAGAACGATTCGCAGTCAATCCTGGCCTGTTAGAA
Inact.1
G G C C G C C

ACATCAGAAGGCTGCAGACAAATATTGGGACAGCTACAGCCATCCCTTCAGACAGGATCAGTAAAGACTTAGATCATTAA
Inact.2
G G C C

TATAATACAGTAGCAACCCTCTATTGTGTACATCAAAGGATAGATGTAAAGACACCAAGGAAGCTTTAGAGAAGATA
Inact.3
C GC C C G

GAGGAAGAGCAAAACAAAGTAAGAAAAAGGCACAGCAAGCAGCAGCTGCAGCTGGCACAGGAAACAGCAGCCAGGTC
Inact.4
GTCC G C G

AGCCAAAATTACCCTATAGTGCAGAACCTACAGGGGCAAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCA

TGGGTAAAAGTAGTAGAAGAAAAGGCTTTCAGCCCAGAAGTAATACCCATGTTTTCAGCATTATCAGAAGGAGCCACC

CCACAGATTATAAACACCATGCTAAACACAGTGGGGGGACATCAAGCAGCCATGCAAATGTTAAAAGAGACTATCAAT
Inact.5
G CC G G T G C

GAGGAAGCTGCAGAAATGGGATAGAGTGCATCCAGTGCATGCAGGGCCTATTGCACCAGGCCAAATGAGAGAACCAAGG

GGAAGTGACATAGCAGGAACCTACTAGTACCCTTCAGGAACAAATAGGATGGATGACAAATAATCCACCTATCCCAGTA

GGAGAAATCTATAAAAGATGGATAATCCTGGGATTAAATAAAATAGTAAGATGTATAGCCCTACCAGCATTCTGGAC
Inact.6
G C G G
Inact.7
G C G C G G

ATAAGACAAGGACCAAAGGAACCCTTTAGAGATTATGTAGACCGGTTCTATAAACTCTAAGAGCGAACAAGCTTCA
T

CAGGATGTAAAAAATTGGATGACAGAAACCTTGTTGGTCCAAAATGCAAACCCAGATTGTAAGACTATTTTAAAGCA
Inact.8
C CC G G T

TTGGGACAGCAGCTACACTAGAAGAAATGATGACAGCATGTCAGGGAGTGGGGGGACCCGGCCATAAGCAAGAGTT
C C C

TTGGCTGAAGCCATGAGCCAAGTAACAAATCCAGCTAACATAATGATGCAGAGAGGCAATTTTAGGAACCAAGAAAG

ACTGTTAAGTGTTCATTGTGGCAAAGAAGGGCACATAGCCAAAAATTGCAGGGCCCTAGGAAAAGGGCTGTTGG

AGATGTGGAAGGGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTAGGGAAGATCTGGCCTTCC

TACAAGGGAAGGCCAGGGAATTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGG

GAGGAGAAAACAACTCCCTCTCAGAAGCAGGAGCCGATAGACAAGGAAGTGTATCCTTTAACTTCCCTCAGATCACTC

TTTGGCAACGACCCCTCGTCACAATAA

FIG. 1

660621 01.03.2000

native HIV-1SF2 gag-protease

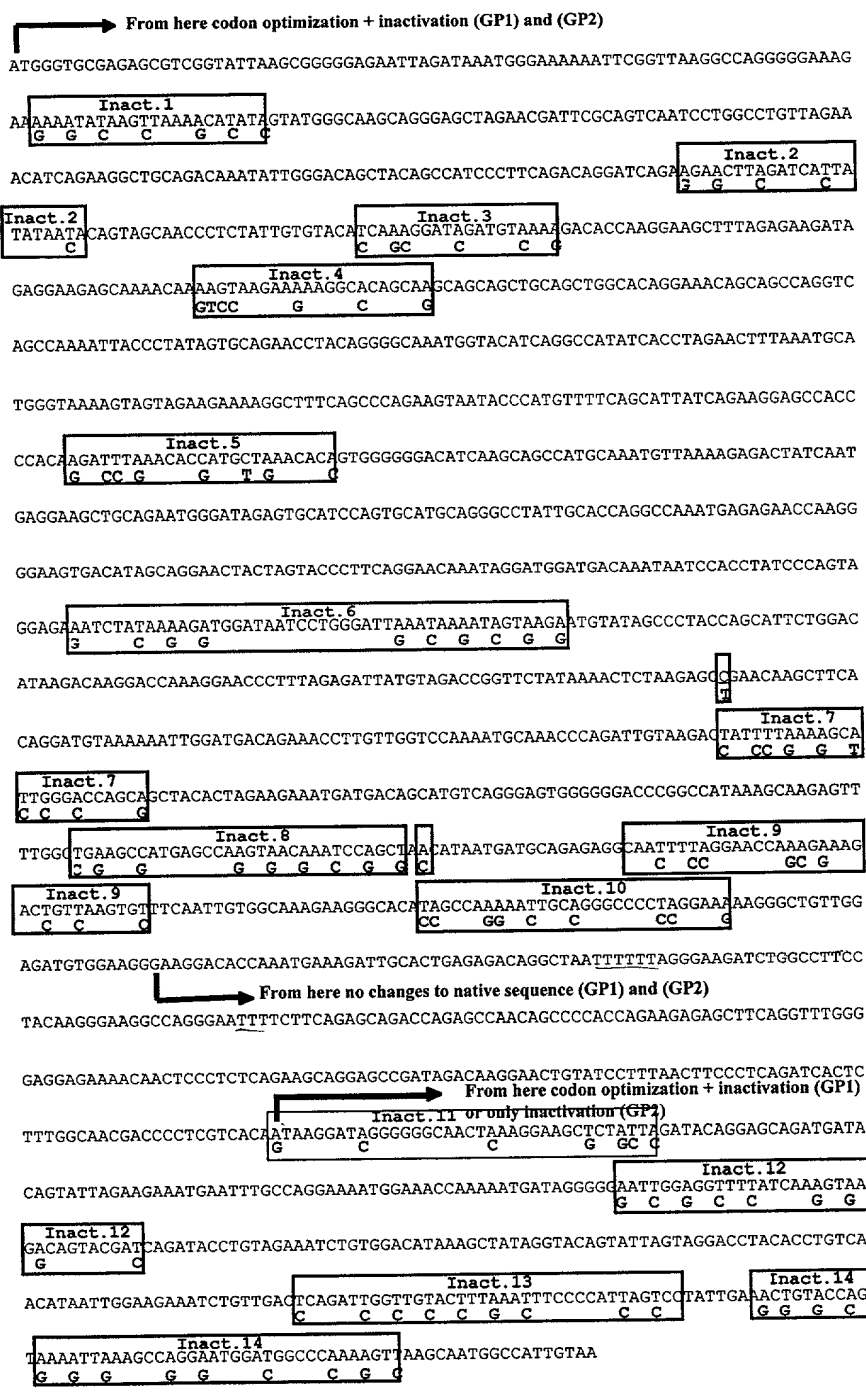


FIG. 2

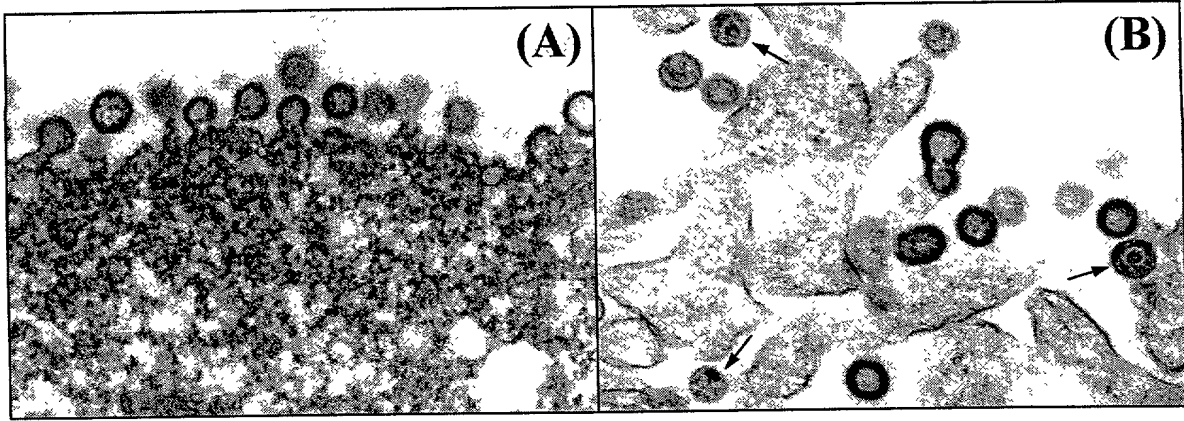


FIGURE 3

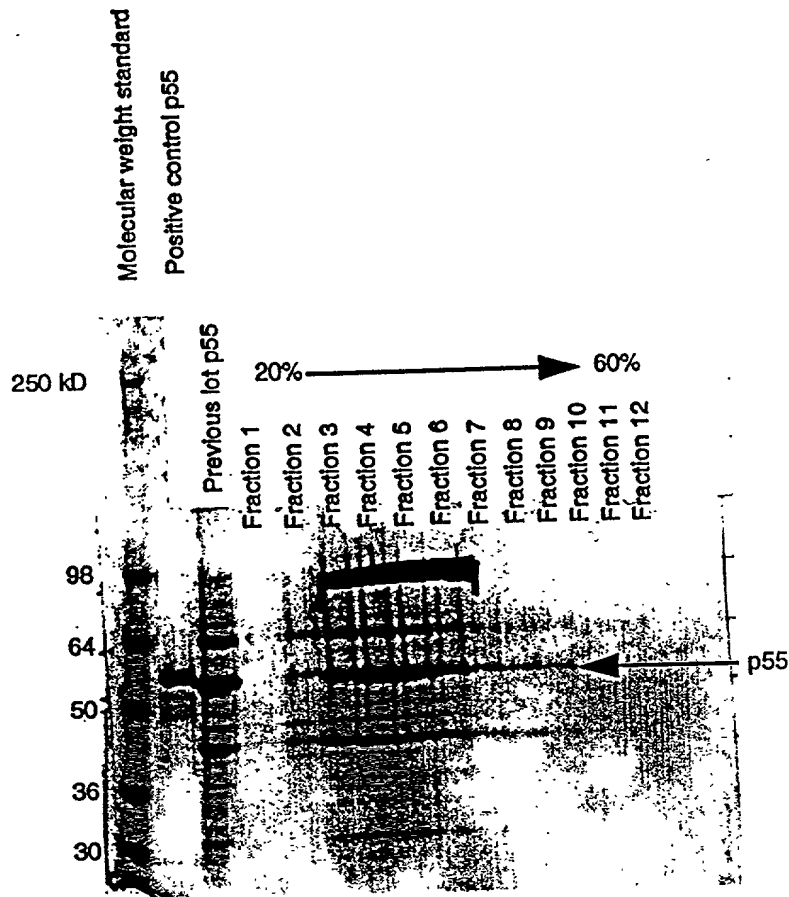


FIG. 4

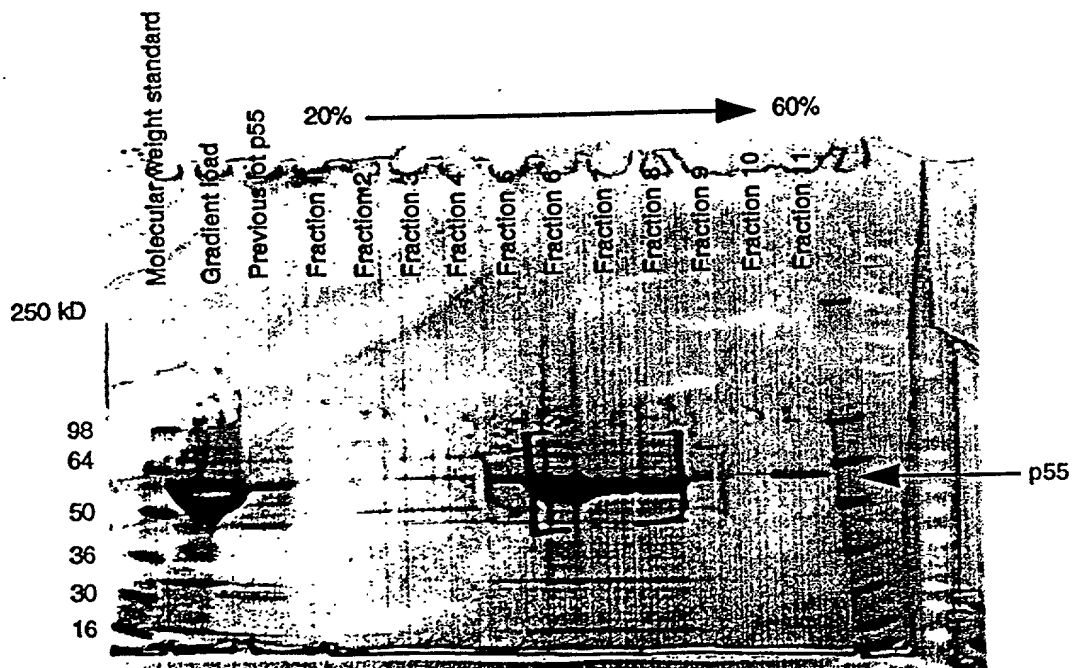


FIG. 5

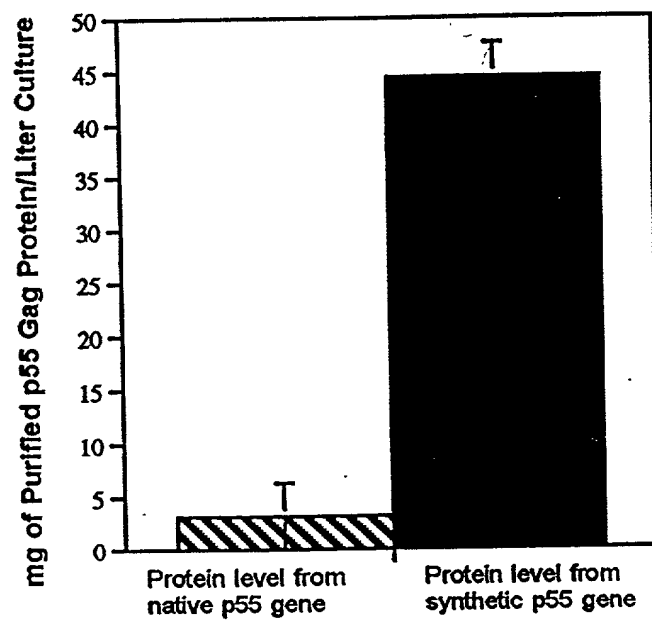


FIG. 6

DNASIS
Multiple Edit3

		10	20	30	40	50	
GagPol.ModSF	1	ATGGGGCGCC	CGCCGAGCGT	CTGAGCGCGC	CGCGAGCTGG	ACAAGTGGGA	50
GagProt.ModS	1	ATGGGGCGCC	CGCCGAGCGT	CTGAGCGCGC	CGCGAGCTGG	ACAAGTGGGA	50
Gag.ModSF2	1	ATGGGGCGCC	CGCCGAGCGT	CTGAGCGCGC	CGCGAGCTGG	ACAAGTGGGA	50
		60	70	80	90	100	
GagPol.ModSF	51	GAAGATCCGC	CTCGGCCCCG	CGGCAAGAA	GAAGTACAG	CTGAAGCACA	100
GagProt.ModS	51	GAAGATCCGC	CTCGGCCCCG	CGGCAAGAA	GAAGTACAG	CTGAAGCACA	100
Gag.ModSF2	51	GAAGATCCGC	CTCGGCCCCG	CGGCAAGAA	GAAGTACAG	CTGAAGCACA	100
		110	120	130	140	150	
GagPol.ModSF	101	TCGTGTGGGC	CAGCCGCGAG	CTGGAGCGCT	TCGCCGTGAA	CCCCGGCCTG	150
GagProt.ModS	101	TCGTGTGGGC	CAGCCGCGAG	CTGGAGCGCT	TCGCCGTGAA	CCCCGGCCTG	150
Gag.ModSF2	101	TCGTGTGGGC	CAGCCGCGAG	CTGGAGCGCT	TCGCCGTGAA	CCCCGGCCTG	150
		160	170	180	190	200	
GagPol.ModSF	151	CTGGAGACCA	CGAGGGGCTG	CGCCAGATC	CTGGGCGAGC	TGCAGCCGAG	200
GagProt.ModS	151	CTGGAGACCA	CGAGGGGCTG	CGCCAGATC	CTGGGCGAGC	TGCAGCCGAG	200
Gag.ModSF2	151	CTGGAGACCA	CGAGGGGCTG	CGCCAGATC	CTGGGCGAGC	TGCAGCCGAG	200
		210	220	230	240	250	
GagPol.ModSF	201	CCTGCAGACC	CGCAGCGAGG	AGCTGCGCAG	CCTGTACAAC	ACCGTGGCCA	250
GagProt.ModS	201	CCTGCAGACC	CGCAGCGAGG	AGCTGCGCAG	CCTGTACAAC	ACCGTGGCCA	250
Gag.ModSF2	201	CCTGCAGACC	CGCAGCGAGG	AGCTGCGCAG	CCTGTACAAC	ACCGTGGCCA	250
		260	270	280	290	300	
GagPol.ModSF	251	CCCTGTACTG	CGTGCACCA	CGCATCGACG	TCAAGGACAC	CAAGGAGGCC	300
GagProt.ModS	251	CCCTGTACTG	CGTGCACCA	CGCATCGACG	TCAAGGACAC	CAAGGAGGCC	300
Gag.ModSF2	251	CCCTGTACTG	CGTGCACCA	CGCATCGACG	TCAAGGACAC	CAAGGAGGCC	300
		310	320	330	340	350	
GagPol.ModSF	301	CTGGAGAGA	TGGAGGAGGA	CGAGAACAAG	TCCAAGAAGA	AGGCCAGCCA	350
GagProt.ModS	301	CTGGAGAGA	TGGAGGAGGA	CGAGAACAAG	TCCAAGAAGA	AGGCCAGCCA	350
Gag.ModSF2	301	CTGGAGAGA	TGGAGGAGGA	CGAGAACAAG	TCCAAGAAGA	AGGCCAGCCA	350
		360	370	380	390	400	
GagPol.ModSF	351	CGCGCGCGCC	CGCGCGCGCA	CGGCAACAG	CAGCCAGGTG	AGCCAGAACT	400
GagProt.ModS	351	CGCGCGCGCC	CGCGCGCGCA	CGGCAACAG	CAGCCAGGTG	AGCCAGAACT	400
Gag.ModSF2	351	CGCGCGCGCC	CGCGCGCGCA	CGGCAACAG	CAGCCAGGTG	AGCCAGAACT	400
		410	420	430	440	450	
GagPol.ModSF	401	ACCCCATCGT	CGAGAACCCTG	CAGGCGCAGA	TGGTGCACCA	CGCCATCAGC	450
GagProt.ModS	401	ACCCCATCGT	CGAGAACCCTG	CAGGCGCAGA	TGGTGCACCA	CGCCATCAGC	450
Gag.ModSF2	401	ACCCCATCGT	CGAGAACCCTG	CAGGCGCAGA	TGGTGCACCA	CGCCATCAGC	450
		460	470	480	490	500	
GagPol.ModSF	451	CGCCGCACCC	TGAACGCGCTG	CGTGAAGGTG	CTGGAGGAGA	AGGCCTTCAG	500
GagProt.ModS	451	CGCCGCACCC	TGAACGCGCTG	CGTGAAGGTG	CTGGAGGAGA	AGGCCTTCAG	500
Gag.ModSF2	451	CGCCGCACCC	TGAACGCGCTG	CGTGAAGGTG	CTGGAGGAGA	AGGCCTTCAG	500
		510	520	530	540	550	
GagPol.ModSF	501	CGCCGAGGTG	ATCCCATGT	TCAGCGCCCT	CAGCGAGGGC	CGCACCCGCC	550
GagProt.ModS	501	CGCCGAGGTG	ATCCCATGT	TCAGCGCCCT	CAGCGAGGGC	CGCACCCGCC	550
Gag.ModSF2	501	CGCCGAGGTG	ATCCCATGT	TCAGCGCCCT	CAGCGAGGGC	CGCACCCGCC	550
		560	570	580	590	600	
GagPol.ModSF	551	CGGACCTGAA	CACGATGTTG	ACACCGTGG	CGGCGCACCA	CGCCGCCATG	600
GagProt.ModS	551	CGGACCTGAA	CACGATGTTG	ACACCGTGG	CGGCGCACCA	CGCCGCCATG	600
Gag.ModSF2	551	CGGACCTGAA	CACGATGTTG	ACACCGTGG	CGGCGCACCA	CGCCGCCATG	600
		610	620	630	640	650	
GagPol.ModSF	601	CAGATGCTGA	AGGAGACCAT	CAACGAGGAG	CGCGCCGAGT	CGGACCCCGT	650
GagProt.ModS	601	CAGATGCTGA	AGGAGACCAT	CAACGAGGAG	CGCGCCGAGT	CGGACCCCGT	650
Gag.ModSF2	601	CAGATGCTGA	AGGAGACCAT	CAACGAGGAG	CGCGCCGAGT	CGGACCCCGT	650
		660	670	680	690	700	
GagPol.ModSF	651	CGACCCCGTG	CAGCCCGGCC	CGATCGCCCC	CGGCCAGATG	CGCGAGCCCC	700
GagProt.ModS	651	CGACCCCGTG	CAGCCCGGCC	CGATCGCCCC	CGGCCAGATG	CGCGAGCCCC	700
Gag.ModSF2	651	CGACCCCGTG	CAGCCCGGCC	CGATCGCCCC	CGGCCAGATG	CGCGAGCCCC	700
		710	720	730	740	750	
GagPol.ModSF	701	CGGCGAGCGA	CATCGCCGCG	ACACCCAGCA	CCCTGCAGGA	CGAGATCGGC	750
GagProt.ModS	701	CGGCGAGCGA	CATCGCCGCG	ACACCCAGCA	CCCTGCAGGA	CGAGATCGGC	750
Gag.ModSF2	701	CGGCGAGCGA	CATCGCCGCG	ACACCCAGCA	CCCTGCAGGA	CGAGATCGGC	750
		760	770	780	790	800	
GagPol.ModSF	751	TGGATGACCA	ACAACCCGCC	CATCCCCGTG	CGCGAGATCT	ACAAGCGGTG	800
GagProt.ModS	751	TGGATGACCA	ACAACCCGCC	CATCCCCGTG	CGCGAGATCT	ACAAGCGGTG	800
Gag.ModSF2	751	TGGATGACCA	ACAACCCGCC	CATCCCCGTG	CGCGAGATCT	ACAAGCGGTG	800

FIG. 7

DNASIS
Multiple Edit3

		810	820	830	840	850	
GagPol.ModSF	801	ATCATCTCTG	GGCTGAACA	AGATCGTGG	ATGTACAGC	CCACCAGCA	850
GagProt.ModS	801	ATCATCTCTG	GGCTGAACA	AGATCGTGG	ATGTACAGC	CCACCAGCA	850
Gag.ModSF2	801	ATCATCTCTG	GGCTGAACA	AGATCGTGG	ATGTACAGC	CCACCAGCA	850
		860	870	880	890	900	
GagPol.ModSF	851	TCTTGGACAT	CGCCAGGGG	CCAGGAGGC	CTTCCGCGA	CTACGTGGAC	900
GagProt.ModS	851	TCTTGGACAT	CGCCAGGGG	CCAGGAGGC	CTTCCGCGA	CTACGTGGAC	900
Gag.ModSF2	851	TCTTGGACAT	CGCCAGGGG	CCAGGAGGC	CTTCCGCGA	CTACGTGGAC	900
		910	920	930	940	950	
GagPol.ModSF	901	CGCTTCTACA	AGACCTGCG	CGCTGAGCAG	CCAGCCAGG	ACGTGAAGAA	950
GagProt.ModS	901	CGCTTCTACA	AGACCTGCG	CGCTGAGCAG	CCAGCCAGG	ACGTGAAGAA	950
Gag.ModSF2	901	CGCTTCTACA	AGACCTGCG	CGCTGAGCAG	CCAGCCAGG	ACGTGAAGAA	950
		960	970	980	990	1000	
GagPol.ModSF	951	CTGGATGACC	SAGACCTGC	TGGTGACAG	CGCCAAACCC	SACTGCAAGA	1000
GagProt.ModS	951	CTGGATGACC	SAGACCTGC	TGGTGACAG	CGCCAAACCC	SACTGCAAGA	1000
Gag.ModSF2	951	CTGGATGACC	SAGACCTGC	TGGTGACAG	CGCCAAACCC	SACTGCAAGA	1000
		1010	1020	1030	1040	1050	
GagPol.ModSF	1001	CCATCCTGAA	CGCTCTCGGC	CCCGCGGCCA	CCCTGGAGGA	SATGATGACC	1050
GagProt.ModS	1001	CCATCCTGAA	CGCTCTCGGC	CCCGCGGCCA	CCCTGGAGGA	SATGATGACC	1050
Gag.ModSF2	1001	CCATCCTGAA	CGCTCTCGGC	CCCGCGGCCA	CCCTGGAGGA	SATGATGACC	1050
		1060	1070	1080	1090	1100	
GagPol.ModSF	1051	CGCTGCCAGG	CGCTGGSCGG	CCCGCGCCAC	AAGGCCCGCG	TGCTGGCCGA	1100
GagProt.ModS	1051	CGCTGCCAGG	CGCTGGSCGG	CCCGCGCCAC	AAGGCCCGCG	TGCTGGCCGA	1100
Gag.ModSF2	1051	CGCTGCCAGG	CGCTGGSCGG	CCCGCGCCAC	AAGGCCCGCG	TGCTGGCCGA	1100
		1110	1120	1130	1140	1150	
GagPol.ModSF	1101	CGCGATGAGC	CAGGTGACGA	ACCCGGCGAC	CATCATGATG	CAGCGCGGCA	1150
GagProt.ModS	1101	CGCGATGAGC	CAGGTGACGA	ACCCGGCGAC	CATCATGATG	CAGCGCGGCA	1150
Gag.ModSF2	1101	CGCGATGAGC	CAGGTGACGA	ACCCGGCGAC	CATCATGATG	CAGCGCGGCA	1150
		1160	1170	1180	1190	1200	
GagPol.ModSF	1151	ACTTCCGCAA	CCAGCGGAG	ACCGTCAAGT	CGTTCAACTG	CGGCAAGGAG	1200
GagProt.ModS	1151	ACTTCCGCAA	CCAGCGGAG	ACCGTCAAGT	CGTTCAACTG	CGGCAAGGAG	1200
Gag.ModSF2	1151	ACTTCCGCAA	CCAGCGGAG	ACCGTCAAGT	CGTTCAACTG	CGGCAAGGAG	1200
		1210	1220	1230	1240	1250	
GagPol.ModSF	1201	GGCCACACCG	CCAGGAACCTG	CCCGCGCCCC	CGCAAGAGG	CGTCTGGCG	1250
GagProt.ModS	1201	GGCCACACCG	CCAGGAACCTG	CCCGCGCCCC	CGCAAGAGG	CGTCTGGCG	1250
Gag.ModSF2	1201	GGCCACACCG	CCAGGAACCTG	CCCGCGCCCC	CGCAAGAGG	CGTCTGGCG	1250
		1260	1270	1280	1290	1300	
GagPol.ModSF	1251	CTGCGGCGCG	SAGGACACCC	AATGAAGA	TTGCACTGAG	AGACAGGCTA	1300
GagProt.ModS	1251	CTGCGGCGCG	SAGGACACCC	AATGAAGA	TTGCACTGAG	AGACAGGCTA	1300
Gag.ModSF2	1251	CTGCGGCGCG	SAGGACACCC	AATGAAGA	TTGCACTGAG	AGACAGGCTA	1300
		1310	1320	1330	1340	1350	
GagPol.ModSF	1301	ATTTTITAGG	SAGATCTGG	CGTTCTTACA	AGGGAGGGCC	AGGGATTTT	1350
GagProt.ModS	1301	ATTTTITAGG	SAGATCTGG	CGTTCTTACA	AGGGAGGGCC	AGGGATTTT	1350
Gag.ModSF2	1301	ATTTTITAGG	SAGATCTGG	CGTTCTTACA	AGGGAGGGCC	AGGGATTTT	1350
		1360	1370	1380	1390	1400	
GagPol.ModSF	1351	CTTCAGAGCA	CGCCAGAGCC	ACAGCCCCA	CCAGAGAGGA	CGTTCAAGTT	1400
GagProt.ModS	1351	CTTCAGAGCA	CGCCAGAGCC	ACAGCCCCA	CCAGAGAGGA	CGTTCAAGTT	1400
Gag.ModSF2	1351	CTTCAGAGCA	CGCCAGAGCC	ACAGCCCCA	CCAGAGAGGA	CGTTCAAGTT	1400
		1410	1420	1430	1440	1450	
GagPol.ModSF	1401	TGGGGAGGAG	AAAACAATC	CGTCTCAGAA	CGAGGAGCCG	ATAGACAAGG	1450
GagProt.ModS	1401	TGGGGAGGAG	AAAACAATC	CGTCTCAGAA	CGAGGAGCCG	ATAGACAAGG	1450
Gag.ModSF2	1401	TGGGGAGGAG	AAAACAATC	CGTCTCAGAA	CGAGGAGCCG	ATAGACAAGG	1450
		1460	1470	1480	1490	1500	
GagPol.ModSF	1451	AACTGTATCC	TTTAACCTTC	CTCAGATCAC	TCCTTGGCAA	CGACCCCTCG	1500
GagProt.ModS	1451	AACTGTATCC	TTTAACCTTC	CTCAGATCAC	TCCTTGGCAA	CGACCCCTCG	1500
Gag.ModSF2	1451	AACTGTATCC	TTTAACCTTC	CTCAGATCAC	TCCTTGGCAA	CGACCCCTCG	1500
		1510	1520	1530	1540	1550	
GagPol.ModSF	1501	TCACAGTAAG	SATCGGCGGG	CAGCTCAAGG	AGGGCGTGCT	CGACACCGGG	1550
GagProt.ModS	1501	TCACAGTAAG	SATCGGCGGG	CAGCTCAAGG	AGGGCGTGCT	CGACACCGGG	1550
Gag.ModSF2	1501	TCACAGTAAG	SATCGGCGGG	CAGCTCAAGG	AGGGCGTGCT	CGACACCGGG	1550
		1560	1570	1580	1590	1600	
GagPol.ModSF	1551	CGCGACGACA	CGCTGCTGGA	CGAGATGAAC	CTGCCCGGCA	ACTGGAGGCC	1600
GagProt.ModS	1551	CGCGACGACA	CGCTGCTGGA	CGAGATGAAC	CTGCCCGGCA	ACTGGAGGCC	1600
Gag.ModSF2	1551	CGCGACGACA	CGCTGCTGGA	CGAGATGAAC	CTGCCCGGCA	ACTGGAGGCC	1600

FIG. 7 (cont'd.)

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[illegible]

FIG. 7 (cont'd.)

DNASIS
Multiple Edit3

		2410	2420	2430	2440	2450	
GagPol.ModSF	2401	GGGGCTTCAC	CACCCCGAC	AAGAAGCACC	AGAAGGAGCC	CCCCCTCCTG	2450
GagProt.ModS	2401	2450
Gag.ModSF2	2401	2450
		2460	2470	2480	2490	2500	
GagPol.ModSF	2451	TGGATGGGCT	ACGAGCTGCA	CCCCGACAAG	TGGACCGTGC	AGCCCATCAT	2500
GagProt.ModS	2451	2500
Gag.ModSF2	2451	2500
		2510	2520	2530	2540	2550	
GagPol.ModSF	2501	GCTGCCCGAG	AAGGACAGCT	GGACCGTGAA	CGACATCCAG	AAGCTGGTGG	2550
GagProt.ModS	2501	2550
Gag.ModSF2	2501	2550
		2560	2570	2580	2590	2600	
GagPol.ModSF	2551	GCAAGCTGAA	CTGGGOCAGC	CAGATCTACG	CCGGCATCAA	GGTGAAGCAG	2600
GagProt.ModS	2551	2600
Gag.ModSF2	2551	2600
		2610	2620	2630	2640	2650	
GagPol.ModSF	2601	CTGTGCAAGC	TGCTGCGGGG	CAOCCAGGCC	CTGACCGAGG	TGATCCCCCT	2650
GagProt.ModS	2601	2650
Gag.ModSF2	2601	2650
		2660	2670	2680	2690	2700	
GagPol.ModSF	2651	GACCGAGGAG	GCCGAGCTGG	AGCTGGCCGA	GAACCGCGAG	ATCCTGAAGG	2700
GagProt.ModS	2651	2700
Gag.ModSF2	2651	2700
		2710	2720	2730	2740	2750	
GagPol.ModSF	2701	AGCCCGTGCA	CGAGGTGTAC	TACGACCCCA	GCAAGGACCT	GGTGGCCGAG	2750
GagProt.ModS	2701	2750
Gag.ModSF2	2701	2750
		2760	2770	2780	2790	2800	
GagPol.ModSF	2751	ATCCAGAAGC	AGGGCCAGGG	CCAGTGGACC	TACCAGATCT	ACCAGGAGCC	2800
GagProt.ModS	2751	2800
Gag.ModSF2	2751	2800
		2810	2820	2830	2840	2850	
GagPol.ModSF	2801	CTTCAAGAAC	CTGAAGACCG	GCAAGTACGC	CCGCATGCGC	GGCGCCACAA	2850
GagProt.ModS	2801	2850
Gag.ModSF2	2801	2850
		2860	2870	2880	2890	2900	
GagPol.ModSF	2851	CCAACGAAGT	GAAGCAGCTG	ACCGAGGCCG	TGCAGAAGGT	GAGCAACGAG	2900
GagProt.ModS	2851	2900
Gag.ModSF2	2851	2900
		2910	2920	2930	2940	2950	
GagPol.ModSF	2901	AGCATCGTGA	TCCTGGGCAA	GATCCCCAAG	TTCAAGCTGC	CCATCCAGAA	2950
GagProt.ModS	2901	2950
Gag.ModSF2	2901	2950
		2960	2970	2980	2990	3000	
GagPol.ModSF	2951	GGAGACCTGG	GAGGCGTGGT	GGATGGAGTA	CTGGCAGGCC	ACCTGGATCC	3000
GagProt.ModS	2951	3000
Gag.ModSF2	2951	3000
		3010	3020	3030	3040	3050	
GagPol.ModSF	3001	CCGAGTGGGA	GTTCGTGAAC	ACCCCCCCCC	TGGTGAAGCT	GTGGTACCAG	3050
GagProt.ModS	3001	3050
Gag.ModSF2	3001	3050
		3060	3070	3080	3090	3100	
GagPol.ModSF	3051	CTGGAGAAGG	AGCCCATCGT	GGGCGCCGAG	ACCTTCTACG	TGGACGGCGC	3100
GagProt.ModS	3051	3100
Gag.ModSF2	3051	3100
		3110	3120	3130	3140	3150	
GagPol.ModSF	3101	CGCCAACCGC	GAGACCAAGC	TGGGCAAGGC	CGGCTACGTG	ACCGAACCGC	3150
GagProt.ModS	3101	3150
Gag.ModSF2	3101	3150
		3160	3170	3180	3190	3200	
GagPol.ModSF	3151	GCCGCCAGAA	GGTGGTGAGC	ATCGCCGACA	CCACCAACCA	GAAGACCGAG	3200
GagProt.ModS	3151	3200
Gag.ModSF2	3151	3200

FIG. 7 (cont'd.)

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DNASTS
Multiple Edit3

		3210	3220	3230	3240	3250	
GagPol.ModSF	3201	CTGCAGGCCA	TCCACCTGGC	CCTGCAGGAC	AGGGGCTGG	AGGTGAACAT	3250
GagProt.ModS	3201	3250
Gag.ModSF2	3201	3250
		3260	3270	3280	3290	3300	
GagPol.ModSF	3251	CGTGACCGAC	AGCCAGTACG	CCCTGGGCAT	CATCCAGGCC	CAGCCCGACA	3300
GagProt.ModS	3251	3300
Gag.ModSF2	3251	3300
		3310	3320	3330	3340	3350	
GagPol.ModSF	3301	AGAGCGAGAG	CGAGCTGGTG	AGCCAGATCA	TCGAGCAGCT	GATCAAGAAG	3350
GagProt.ModS	3301	3350
Gag.ModSF2	3301	3350
		3360	3370	3380	3390	3400	
GagPol.ModSF	3351	GAGAAGGTGT	ACCTGGCCTG	GGTGCCCGCC	CACAAGGGCA	TCGGCGGCAA	3400
GagProt.ModS	3351	3400
Gag.ModSF2	3351	3400
		3410	3420	3430	3440	3450	
GagPol.ModSF	3401	CGAGCAGGTG	GACAAGCTGG	TGAGCGCCGG	CATCCGCAAG	GTGCTGTTC	3450
GagProt.ModS	3401	3450
Gag.ModSF2	3401	3450
		3460	3470	3480	3490	3500	
GagPol.ModSF	3451	TGAACGGCAT	CGACAAGGCC	CAGGAGGAGC	ACGAGAAGTA	CCACAGCAAC	3500
GagProt.ModS	3451	3500
Gag.ModSF2	3451	3500
		3510	3520	3530	3540	3550	
GagPol.ModSF	3501	TGGCGCGCCA	TGGCCAGCGA	CTTCAACCTG	CCCCCGTGG	TGGCCAAGGA	3550
GagProt.ModS	3501	3550
Gag.ModSF2	3501	3550
		3560	3570	3580	3590	3600	
GagPol.ModSF	3551	GATCGTGGCC	AGCTGCGACA	AGTGCCAGCT	GAAGGGCGAG	GCCATGCACG	3600
GagProt.ModS	3551	3600
Gag.ModSF2	3551	3600
		3610	3620	3630	3640	3650	
GagPol.ModSF	3601	GCCAGGTGGA	CTGCAGCCCC	GGCATCTGGC	AGCTGGACTG	CACCCACCTG	3650
GagProt.ModS	3601	3650
Gag.ModSF2	3601	3650
		3660	3670	3680	3690	3700	
GagPol.ModSF	3651	GAGGGCAAGA	TCATCTGGT	GGCCGTGCAC	GTGGCCAGCG	GCTACATCGA	3700
GagProt.ModS	3651	3700
Gag.ModSF2	3651	3700
		3710	3720	3730	3740	3750	
GagPol.ModSF	3701	GGCCGAGGTG	ATCCCCGCGG	AGACCGGCCA	GGAGACCGGC	TACTTCCTGC	3750
GagProt.ModS	3701	3750
Gag.ModSF2	3701	3750
		3760	3770	3780	3790	3800	
GagPol.ModSF	3751	TGAAGCTGGC	CGGCGGCTGG	CCCGTGAAGA	CCATCCACAC	CGACAACGGC	3800
GagProt.ModS	3751	3800
Gag.ModSF2	3751	3800
		3810	3820	3830	3840	3850	
GagPol.ModSF	3801	AGCAACTTCA	CCAGCACCAC	CGTGAAGGCC	GCCTGCTGGT	GGGCGGGCAT	3850
GagProt.ModS	3801	3850
Gag.ModSF2	3801	3850
		3860	3870	3880	3890	3900	
GagPol.ModSF	3851	CAAGCAGGAG	TTCGGCATCC	CCTACAACCC	CCAGAGCCAG	GGCGTGGTGG	3900
GagProt.ModS	3851	3900
Gag.ModSF2	3851	3900
		3910	3920	3930	3940	3950	
GagPol.ModSF	3901	AGAGCATGAA	CAACGAGCTG	AAGAAGATCA	TCGGCCAGGT	GCGCGACCAG	3950
GagProt.ModS	3901	3950
Gag.ModSF2	3901	3950
		3960	3970	3980	3990	4000	
GagPol.ModSF	3951	GCCGAGCAAC	TGAAGACCGC	CGTGCAAGATG	GCCGTGTTC	TCCACAACCTT	4000
GagProt.ModS	3951	4000
Gag.ModSF2	3951	4000

FIG. 7 (cont'd.)

DNASIS
Multiple Edit3

		4010	4020	4030	4040	4050	
GagPol.ModSF	4001	CAAGCGCAAG	GGGGCATCG	GCGGCTACAG	CGCCGGCGAG	CGCATCGTGG	4050
GagProt.ModS	4001	4050
Gag.ModSF2	4001	4050
		4060	4070	4080	4090	4100	
GagPol.ModSF	4051	ACATCATCGC	CACCGACATC	CAGACCAAGG	AGCTGCAGAA	GCAGATCACC	4100
GagProt.ModS	4051	4100
Gag.ModSF2	4051	4100
		4110	4120	4130	4140	4150	
GagPol.ModSF	4101	AAGATCCAGA	ACTTCGCGGT	GTACTACCGC	GACAACAAGG	ACCCCCCTGTG	4150
GagProt.ModS	4101	4150
Gag.ModSF2	4101	4150
		4160	4170	4180	4190	4200	
GagPol.ModSF	4151	GAAGGGCCCG	GCCAAGCTGC	TGTGGAAGGG	CGAGGGCGCC	GTGGTGATCC	4200
GagProt.ModS	4151	4200
Gag.ModSF2	4151	4200
		4210	4220	4230	4240	4250	
GagPol.ModSF	4201	AGGACAACAG	CGACATCAAG	GTGGTGCCCC	GCCGCAAGGC	CAAGATCATC	4250
GagProt.ModS	4201	4250
Gag.ModSF2	4201	4250
		4260	4270	4280	4290	4300	
GagPol.ModSF	4251	CGGACTACG	GCAAGCAGAT	GGCCGGGAC	GACTGGGTGG	CCAGCCGCCA	4300
GagProt.ModS	4251	4300
Gag.ModSF2	4251	4300
		4310	4320	4330	4340	4350	
GagPol.ModSF	4301	GGACGAGGAC	TAG.....	4350
GagProt.ModS	4301	4350
Gag.ModSF2	4301	4350

FIG. 7 (cont'd.)

650227 "GFS" 460

660327 61552460

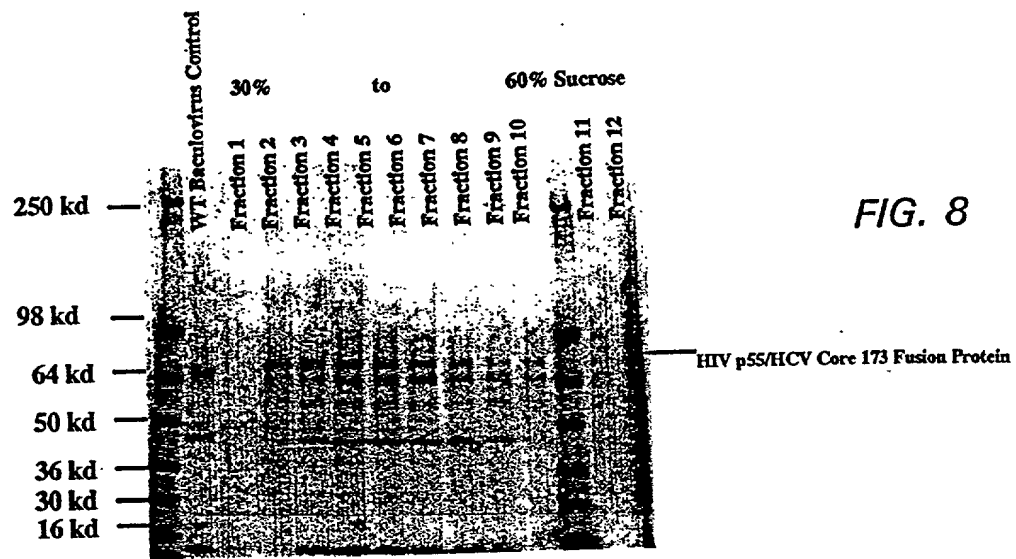


FIG. 8

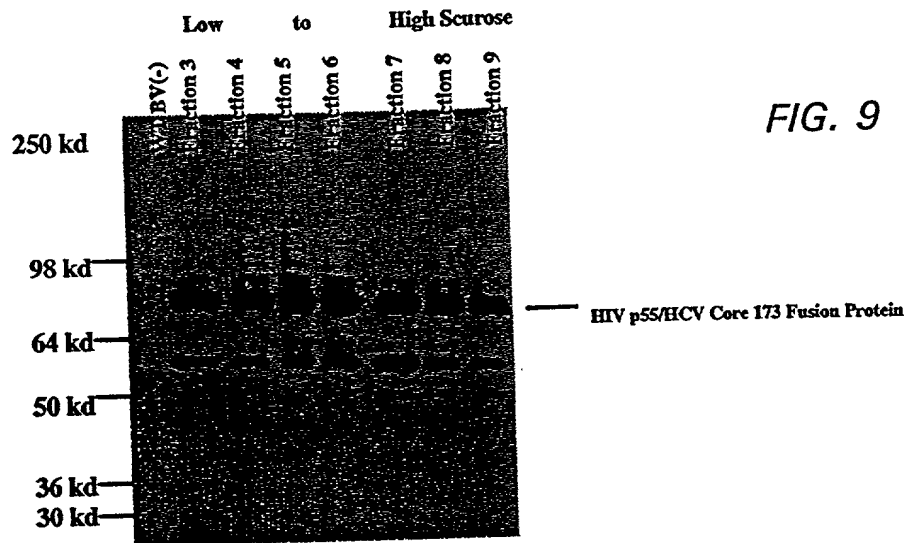


FIG. 9

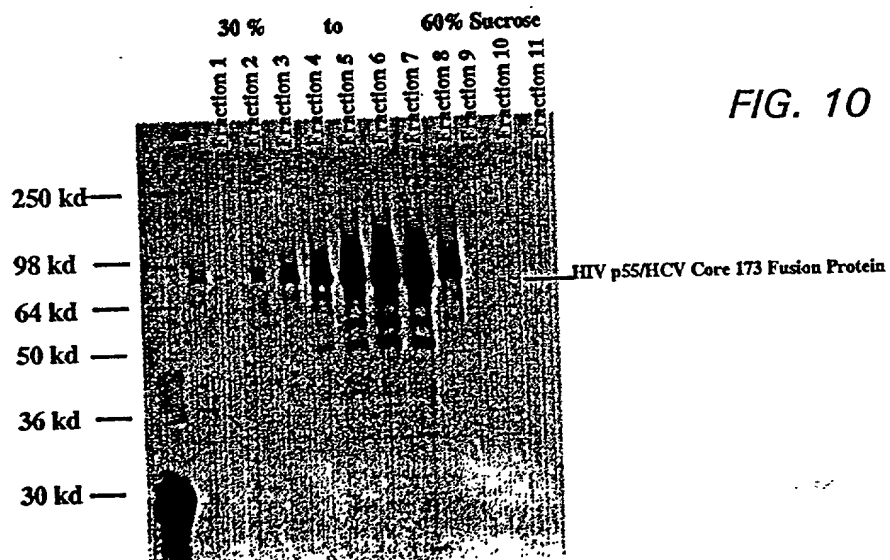


FIG. 10

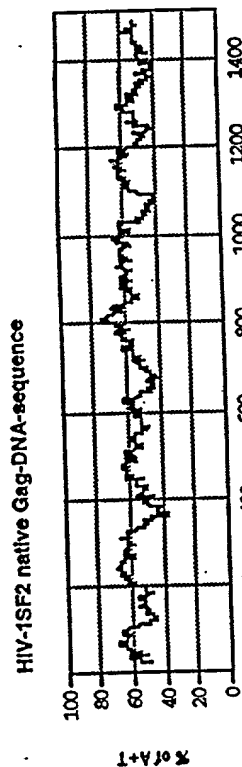


FIG. 11B

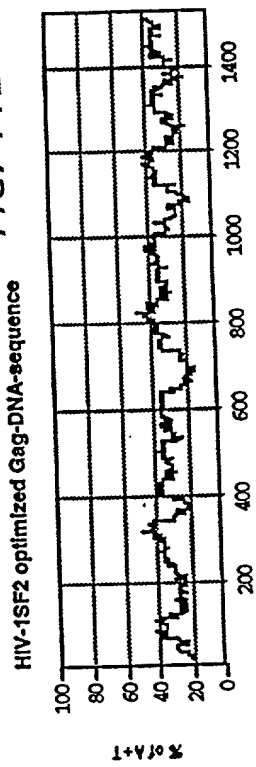


FIG. 11D

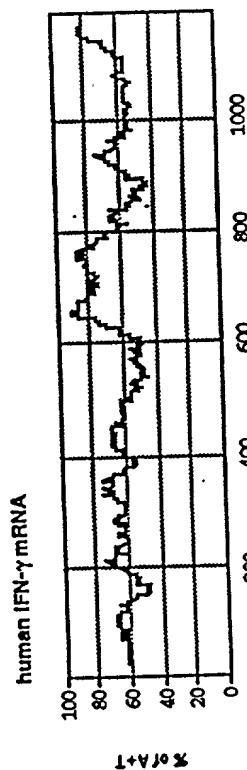


FIG. 11A

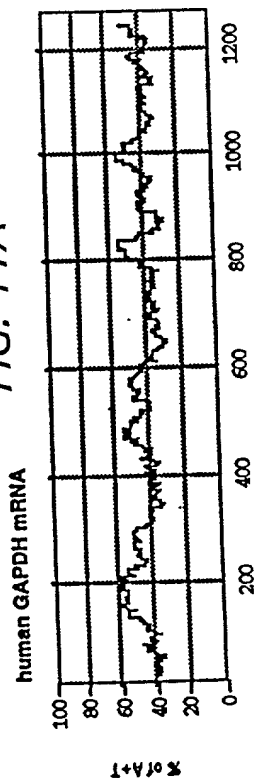


FIG. 11C

native HIV-1SF2 gag-polymerase

ATGGGTGCGAGAGCGCTCGGTATTAAAGCGGGGAGAATTAGATAAATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAAG

Inact. 1
AAAAATATAAGTTAAACATATATATGGGCAAGCAGGGAGCTAGAACGATTCCGAGTCAATCCTGGCCTGTTAGAA

Inact. 2
ACATCAGAAGGCTGCAGACAAATATTGGGACAGCTACAGCCATCCCTTCAGACAGGATCAGAACTTATGATCATTA

Inact. 3
TATAATACTAGTAGCAACCTCTATTGTGTACATCAAAAGGATAGATGTAAATGACACCAAGSAGCTTTAGAGAAGATA

Inact. 4
GAGGAAGAGCAAAACAAAGTAAGAAAAAGGCACAGCAATCAGCAGCTGCAGCTGGCACAGGAACAGCAGCCAGGTC

Inact. 5
AGCCAAAATTACCTTATAGTCGAGAACCTACAGGGGCAATGGTACATCAGGCCATATCACCTAGAACTTTAAATGCA

Inact. 6
TGGGTAAAAGTAGTAGAAGAAAAGGCTTTCAGCCAGAAATATACCATGTTTTCAGCATTATCAGAAGGAGCCACC

Inact. 7
CCACAGATTATAACCATGCTTAAACACATGTGGGGGACATCAAGCAGCCATGCAATGTTAAAGAGACTATCAAT

Inact. 8
GAGGAAGCTGCAGAAATGGATAGAGTGATCCAGTGATGCAGGGCTATTGCAACAGGCCAAATGAGAGAACCAAGG

Inact. 9
GGAAGTGACATAGCAGGAATCTAGTACCTTCAGGAACAAATAGGATGGATGACAAATATCCACCTATCCAGTA

Inact. 10
GGAGTAATCTATAAAGATGGATAATCTGGGATTAAATAAATAGTAAGATGTATAGCCCTACAGCATTCTGGAC

Inact. 11
ATAAGCAAGGACCAAGGAACCTTTAGAGATTATGTAGACCGGTTCTATAAACTCTAAGAGCAACCAAGCTTCA

Inact. 12
CAGGATGTAAAAAATTTGGATGACAGAAACCTTGTGGTCCAAAATGCAACCCAGATTGTAAAGATTTTAAAGCA

Inact. 13
TTGGGACGAGCACTACACTAGAAGAAATGATGACAGCATGTCAGGGAGTGGGGGACCCGCCATAAAGCAAGATT

Inact. 14
TTGGCTGAGGCCATGAGCCAGTACCAATCCAGCTAAATAATGATGCAGAGAGGAATTTTAGGAACCAAGAAAG

Inact. 15
ACTGTTAAGTGTCTCAATTGTGGCAAGAAGGGGCACATAGCCAAAATGTCAGGGGCCCTAGGAAGAGGGCTGTGG

Inact. 16
AGATGTGGAAGGGAAGGACACCAATGAAGATTGCACTGAGAGACAGGCTAATTTTTAGGGAAGATCTGGCCTTCC

Inact. 17
TACAAGGGAAGGCCAGGAATTTCTTCAGAGCAGACAGGCCAACGCCCCACAGAGAGAGCTTCAGGTTTGGG

Inact. 18
GAGGAGAAAACACTCCCTCTCAGAACGAGGACCGATAGACAGGAAGTGTATCCTTTAACTTCCCTCAGATCACTC

Inact. 19
TTTGGCAAGCAGCCCTCGTCACTAAGGATAGGGGGCACTAAAGGAAGCTTATTATACAGGAGCAGATGATA

Inact. 20
CAGTATTAGAAGAAATGAATTTGCCAGGAAAATGGAACCAAAAATGATAGGGGGAATTTGGAGGTTTATCAAGTAA

Inact. 21
CACAGTACGATAGATACCTGTAGAAATCTGTGGACATAAAGCTATAGGTACAGTATTAGTAGGAACCTACACCTGTCA

Inact. 22
ACATAATTTGGAAGAAATCTGTGTAGCTCAGATTGGTTGTACTTTAAATTTCCCAATTAGTCCATTTGAACTGTACCA

Inact. 23
TAAATTAAGGCCAAGATGGATGGGCCAAAAGTTAAGCAATGGCCATTGACAGAAAGAAAAATAAAGCATTAGTAG

Inact. 24
AGATATGTACAGAAATGGAAGGAAAGGGAATTTCAAAATTTGGGCTGAAATCCATACAACTACTCCAGTATTGT

Inact. 25
CTATAAAGAAAAAGCAGTACTAAATGCGAGAAACTAGTAGATTTCAGAGAACTTAATAAAGAACTCAAGCTTCT

Inact. 26
GGGAAGTTCAGTTAGGAATACCAACCCCGAGGGTTAAAGAAAGAAATCAGTACAGTATTGGATGTGGGTGATG

Inact. 27
CATACTTTTCAGTTCCCTTAGATAAAGACTTTAGAAAGTATAGTCACTTTACCATCTAGTATAAACATGAGACAC

Inact. 28
CAGGATTAGATATCAGTACAACTGTCTGCCACAGGGATGGAAGGATCAACAGCAATATCCAAAGTAGCATGACAA

Inact. 29
AAATCTTAGAGCCTTTTAGMAAACAGAAATCCAGACATAGTTATCTATCAATACATGGATGATTGTATGTAGGATCTG

Inact. 30
ACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAAGTGAAGACAGCATCTGTTGAGGTGGGATTACCAACCCAG

Inact. 31
ACAAAACATCAGAAAGAACTCCATCTCTTGGATGGGTTATGAATCCATCTGATAAATGGACAGTACAGCTTA

Inact. 32
TAACTGCTGCCAGAAAAGACAGCTGGACTGTCAATGACATACAGAAAGTATGTTGGGAAAATGAATTTGGGCAAGTCAGA

Inact. 33
TTTATGCGAGGATTAAGTAAGCAGTTATGTAACTCCTTAGAGGAACCAAGCACTAACAGAAATTAATACCACTAA

Inact. 34
CAGAAAGACGACAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAAGAACAGTACATGAAGTATATTATGACCCAT

Inact. 35
CAAAAGACTTAGTAGCAGAAATACAGAACGAGGGCAAGGCCAATGGACATATCAATTTATCAAGGCCATTTAAAT

Inact. 36
ATCTGAAAACAGGAAGTATGCAAGGATGAGGGGTGCCACACTAATGATGTAAACAGTTAACAGAGGCAGTGCAAA

Inact. 37
AAGTATCCACAGAAAGCATAGTAATATGGGGAAGATTCTTAAATTTAACTACCAATACAAAGGAACATGGGAAG

Inact. 38
CATGGTGGATGGAGTATTGGCAAGCTACCTGGATTCTGAGTGGGAGTTGTCAATACCCCTCCCTTAGTGAAATTAT

Inact. 39
GGTACAGTTAGAGAAAGAACCATAGTAGGAGCAGAACTTTCTATGTAGTGGGGCAGCTAATAGGGAGACTAAAT

Inact. 40
TAGGAAAGCAGGATATGTACTGTGACAGAGGAACAAAAAGTTGTCTCCTAGCTGACACAAACATCAGAAAGACTG

Inact. 41
AATTCAAGCAATTCATCTAGCTTTTCAGGATTTCGGGATTAGAAATTAACATAGTACAGACTCAATATGCATTAG

Inact. 42
GAATCTTCAAGCAACACAGATAAGAGTGAATCAGAGTTAGTCACTCAATATAAGAGCAGTTAATAAAGGAAAG

Inact. 43
AGCTCTACCTGGCATGGTACCCAGACACAAAGGAATTGGAGGAATGAACAGTAGATAAATAGTCACTGCTGGAA

Inact. 44
TCAGGAAGTACTATTGTAATGGAAATAGATAAGGCCAAGAAACATAGAAATATCAGTAATTTGGAGAGCAA

Inact. 45
TGCTAGTGATTTTAACTGCCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAATGTCACTTAAAGGAG

Inact. 46
AAGCCATGCTAGGCAAGTACAGTGTAGTCCAGGAATATGCAACTAGATTGTACATCTAGAAAGGAATAATATCC

Inact. 47
TGGTAGCAGTTTATGTAGTACAGTGGATATATAGAAAGCAGAAATTTTCCAGCAGAGACAGGGCAGGAACAGCATATT

Inact. 48
TTCTCTTAAATTTAGCAGGAAGATGGCCAGTAAAAACATATACAGACAAATGGCAGCAATTTCCACGACTACTCGG

Inact. 49
TTAAGGCGCGCTTGTGGTGGGAGGATCAAGCAGGAATTTGGCATTCCCTACAATCCCAAGTCAAGGAGTAGTAG

Inact. 50
AATCTATGAATTAATGAATTAAGAAAAATATAGGACAGGTAAAGAGATCAGGCTGAACACCTTAAGACAGCAGTACAAA

Inact. 51
TGGCAGATTTCATCCCAATTTTAAAGAAAAAGGGGGGATTGGGGGATACAGTGCAGGGGAAAGAAATAGTAGACATTA

Inact. 52
TAGCAACAGACATACAACTTAAGAACTACAAAGCAAAATACAAAATTTCAAAATTTTGGGTTTATTACAGGGACA

Inact. 53
ACAAAGATCCCTTTGGAAGAGCAAGCAAGCTTCTCTGGAAGGTGAAGGGGCAAGTAGTAATACAAAGATAATAGTG

Inact. 54
ACATAAAGATAGTGCAGAAAGAAAGCAAAATCATTAGGGATTATGGAAGACAGATGCAGGTGATGATTGTGTGG

Inact. 55
CAAGTAGACAGGATGAGGATTAG

FIG. 12

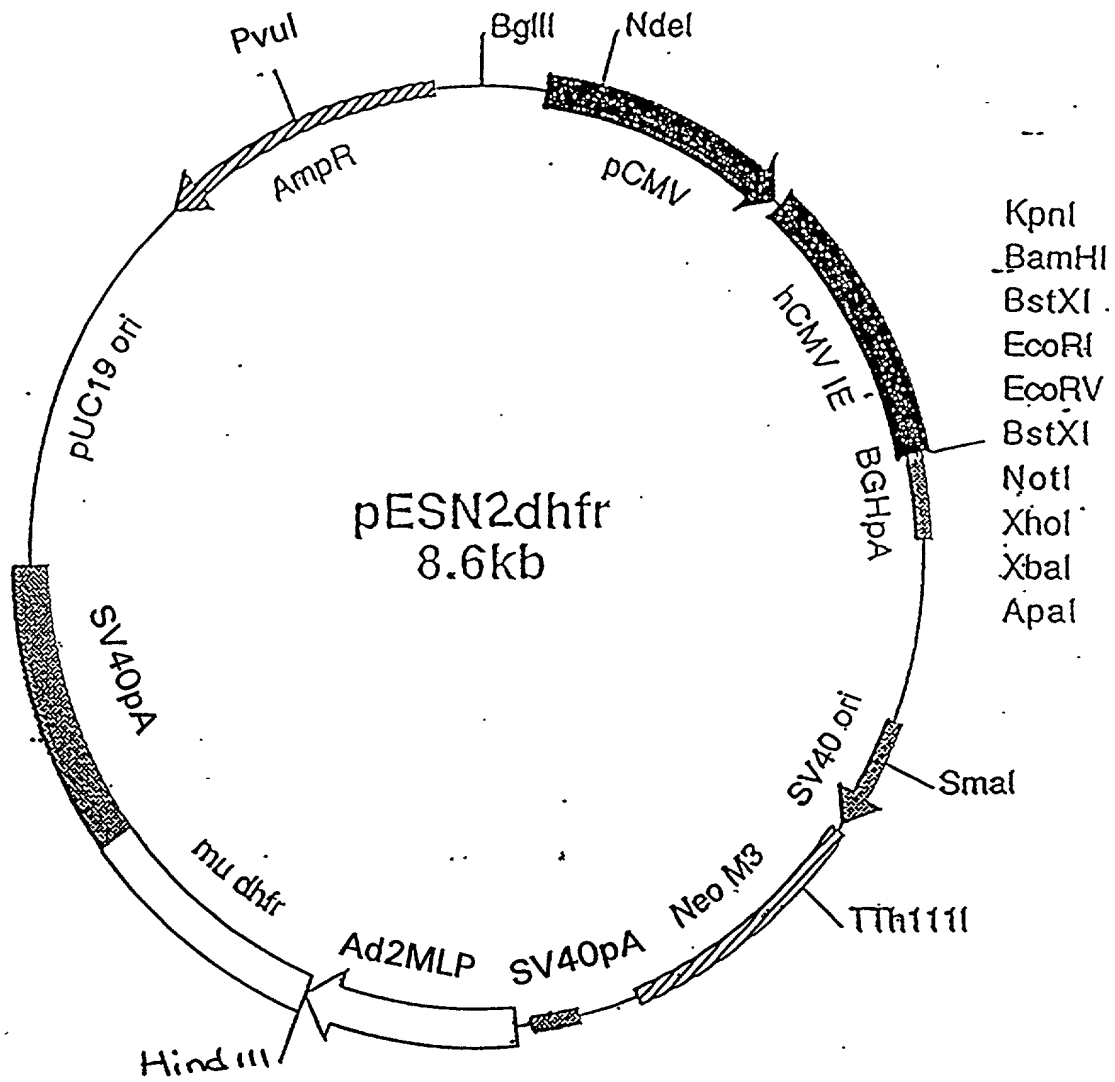


FIG. 13A

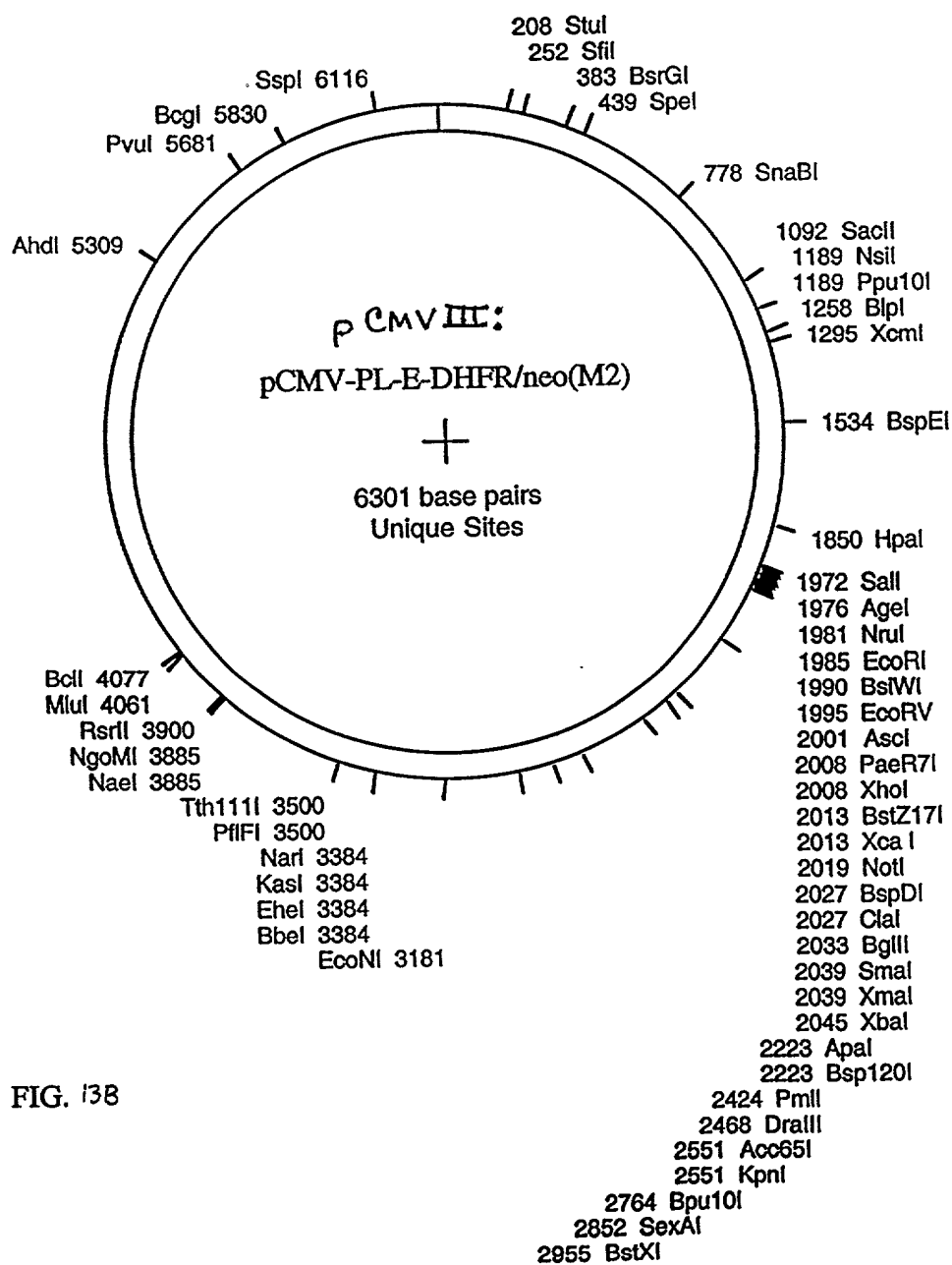


FIG. 138

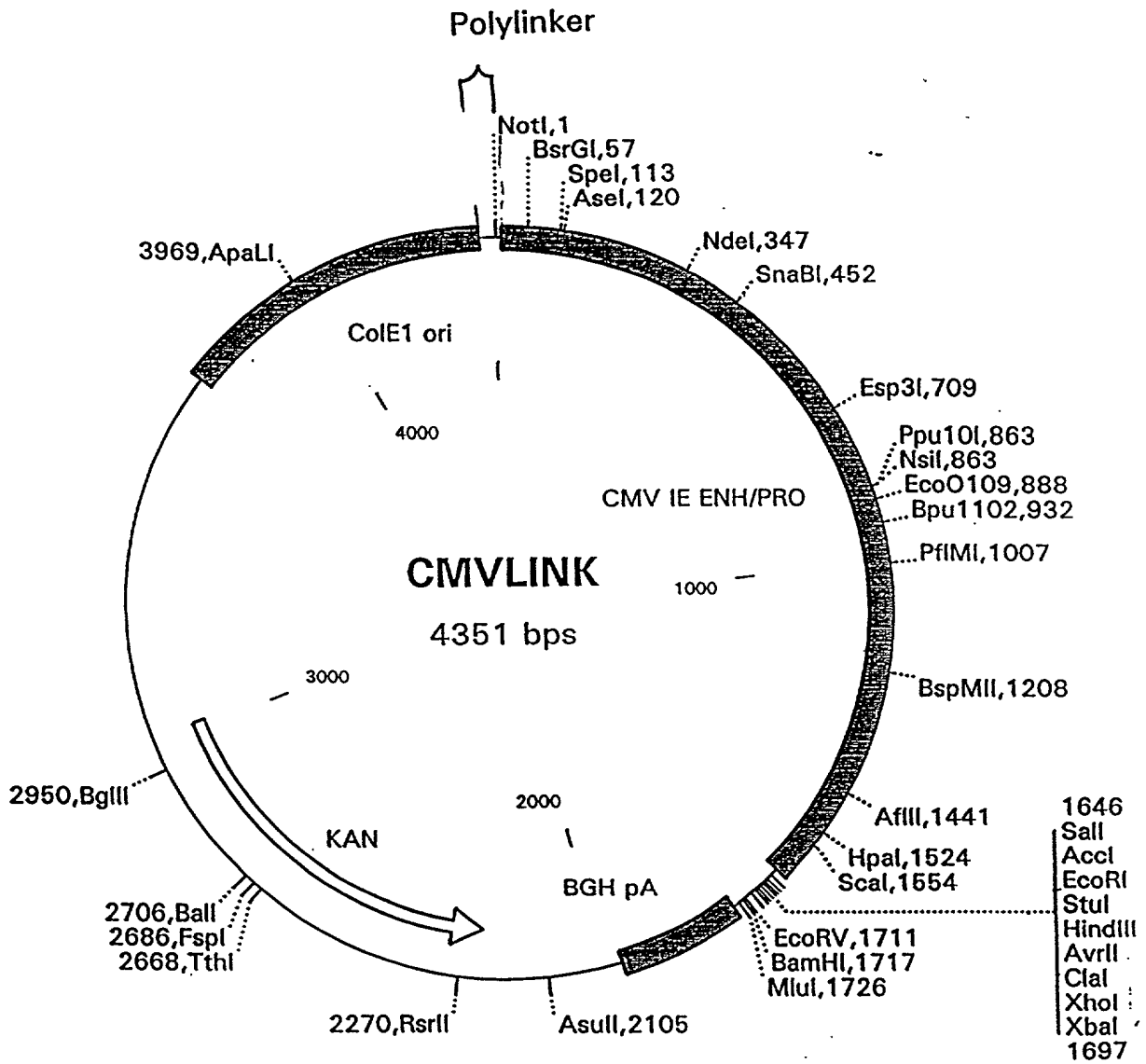


FIG.14

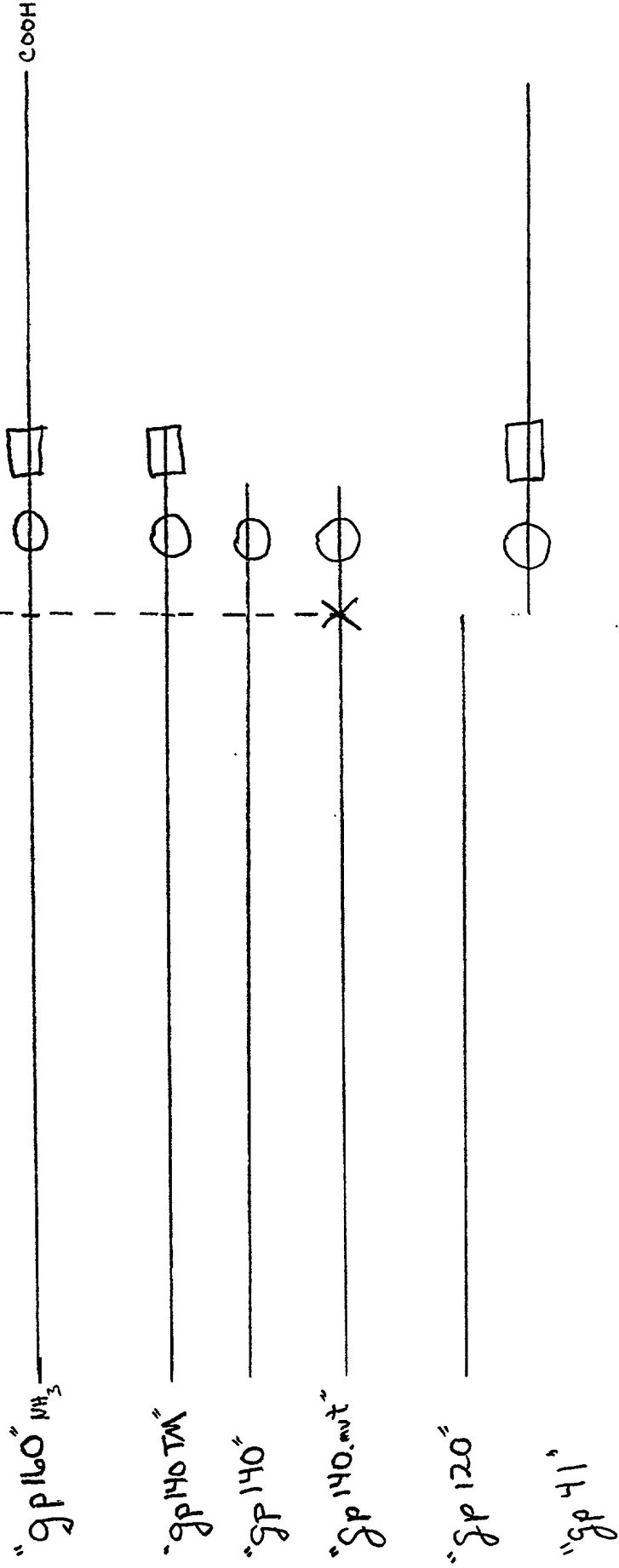


FIG. 15

gp120wtSF162

GTAGAAAAATTGTGGGTACAGTCTATTATGGGGTACCTGTGTGGAAAGAAGCAACCACCACTCTATTTT
GTGCATCAGATGCTAAAGCCTATGACACAGAGGTACATAATGTCTGGGCCACACATGCCTGTGTACCCAC
AGACCTTAACCCACAAGAAATAGTATTGGAAATGTGACAGAAAATTTTAACATGTGGAAAAATAACATG
GTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGTCTAAAGCCATGTGTAAAAGTTAAACCC
CACTCTGTGTTACTCTACATTGCACTAATTTGAAGAATGCTACTAATACCAAGAGTAGTAATTGGAAAAGA
GATGGACAGAGGAGAAAATAAAAAATTGCTCTTTCAAGGTCACCACAAGCATAAGAAATAAGATGCAGAAA
GAATATGCACTTTTTTATAAACTTGATGTAGTACCAATAGATAATGATAATACAAGCTATAAAATGATAA
ATTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAACCAATCCCATACATTATTG
TGCCCCGGCTGGTTTTGCGATTCTAAAGTGTAATGATAAGAAGTTCAATGGATCAGGACCATGTACAAAT
GTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTGTCAACTCAATTGCTGTTAAATGGCAGTC
TAGCAGAAGAAGGGGTAGTAATTAGATCTGAAAATTTACAGACAATGCTAAAACATAATAGTACAGCT
GAAGGAATCTGTAGAAATTAATTGTACAAGACCTAACAAATAATACAAGAAAAAGTATAACTATAGGACCG
GGGAGAGCATTTTATGCAACAGGAGACATAATAGGAGATATAAGACAAGCACATTGTAACATTAGTGGAG
AAAAATGGAATAACACTTTAAACAGATAGTTACAAAATTACAAGCACAAATTTGGGAATAAAACAATAGT
CTTTAAGCAATCCTCAGGAGGGGACCCAGAAATTGTAATGCACAGTTTTTAATTGTGGAGGGGAATTTTTC
TACTGTAATTC AACACAGCTTTTTAATAGTACTTGGAATAATACTATAGGGCCAAATAACACTAATGGAA
CTATCACACTCCCATGCAGAATAAAACAAATTATAAACAGGTGGCAGGAAGTAGGAAAAGCAATGTATGC
CCCTCCCATCAGAGGACAAATTAGATGCTCATCAAATATTACAGGACTGCTATTAACAAGAGATGGTGGT
AAAGAGATCAGTAACACCACCGAGATCTTCAGACCTGGAGGTGGAGATATGAGGGACAATTGGAGAAGTG
AATTATATAAATATAAAGTAGTAAAAATTGAGCCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGT
GGTGCAGAGAGAAAAAAGA

FIG. 16 (SEQ ID NO:30)

gp140wtSF162

GTAGAAAAATTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAGCAACCACCCTCTATTTT
GTGCATCAGATGCTAAAGCCTATGACACAGAGGTACATAATGTCTGGGCCACACATGCCCTGTGTACCCAC
AGACCCTAACCCACAAGAAATAGTATTGGAAAATGTGACAGAAAATTTTAACATGTGGAAAAATAACATG
GTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGTCTAAAGCCATGTGTAAAGTTAACCC
CACTCTGTGTTACTCTACATTGCACTAATTTGAAGAATGCTACTAATACCAAGAGTAGTAATTGGAAAGA
GATGGACAGAGGAGAAATAAAAAATTGCTCTTTCAAGGTCACCACAAGCATAAGAAATAAGATGCAGAAA
GAATATGCACCTTTTTTATAAACTTGATGTAGTACCAATAGATAATGATAATACAAGCTATAAATTGATAA
ATTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAACCAATTCCCATACATTTATG
TGCCCCGGCTGGTTTTGCGATTCTAAAGTGTAATGATAAGAAGTTCAATGGATCAGGACCATGTACAAAT
GTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTGTCAACTCAATTGCTGTAAATGGCAGTC
TAGCAGAAGAAGGGGTAGTAATTAGATCTGAAAATTTACAGACAATGCTAAAACTATAATAGTACAGCT
GAAGGAATCTGTAGAAATTAAATTGTACAAGACCTAACAAATAACAAGAAAAAGTATAACTATAGGACCG
GGGAGAGCATTTTTATGCAACAGGAGACATAATAGGAGATATAAGACAAGCACATTGTAACATTAGTGGAG
AAAAATGGAATAACACTTTTAAACAGATAGTTACAAAATTACAAGCACAATTTGGGAATAAAACAATAGT
CTTTAAGCAATCCTCAGGAGGGGACCCAGAAATTGTAATGCACAGTTTAAATTGTGGAGGGGAATTTTTTC
TACTGTAATTTCAACACAGCTTTTTTAATAGTACTTTGGAATAATACTATAGGGCCAAATAACACTAATGGAA
CTATCACACTCCCATGCAGAATAAAACAAATTATAAACAGGTGGCAGGAAGTAGGAAAAGCAATGTATGC
CCCTCCCATCAGAGGACAAATTAGATGCTCATCAAATATTACAGGACTGCTATTAACAAGAGATGGTGGT
AAAGAGATCAGTAACACCACCGAGATCTTCAGACCTGGAGGTGGAGATATGAGGGACAATTGGAGAAGTG
AATTATATAAATATAAAGTAGTAAAAATTGAGCCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGT
GGTGCAGAGAGAAAAAAGAGCAGTGACGCTAGGAGCTATGTTCTTGGGTTCTTGGGAGCAGCAGGAAGC
ACTATGGGCGCACGGTCACTGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAACAGC
AGAACAATTTGCTGAGAGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCA
GCTCCAGGCAAGAGTCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGGGATTTGGGGTTGC
TCTGGAAAACCTATTTGCACCACTGCTGTGCCCTTGAATGCTAGTTGGAGTAATAAATCTCTGGATCAGA
TTTGGAAATAACATGACCTGGATGGAGTGGGAGAGAGAAATTGACAATTACACAACTTAATATACACCTT
AATTGAAGAATCGCAGAACCAACAAGAAAAGAAATGAACAAGAATTATTAGAATTGGATAAGTGGGCAAGT
TTGTGGAATTGGTTTGACATATCAAATGGCTGTGGTATATA

FIG. 17 (SEQ ID NO:31)

gp160wtSF162

GTAGAAAAATTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAGCAACCACCACTCTATTTT
GTGCATCAGATGCTAAAGCCTATGACACAGAGGTACATAATGTCTGGGCCACACATGCCTGTGTACCCAC
AGACCCTAACCACAAGAAATAGTATTGGAAAATGTGACAGAAAATTTTAACATGTGGAAAAATAACATG
GTAGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGTCTAAAGCCATGTGTAAAGTTAACCC
CACTCTGTGTTACTCTACATTGCACTAATTTGAAGAATGCTACTAATACCAAGAGTAGTAATTGGAAAAGA
GATGGACAGAGGAGAAAATAAAAAATTGCTCTTTCAAGGTCAACACAAGCATAAGAAATAAGATGCAGAAA
GAATATGCACCTTTTTTATAAACTTGATGTAGTACCAATAGATAATGATAATACAAGCTATAAATTGATAA
ATTGTAACACCTCAGTCATTACACAGGCCTGTCCAAAGGTATCCTTTGAACCAATTCCCATACATTATTG
TGCCCCGGCTGGTTTTGCGATTCTAAAGTGTAATGATAAGAAGTTCAATGGATCAGGACCATGTACAAAT
GTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTGTCAACTCAATTGCTGTTAAATGGCAGTC
TAGCAGAAGAAGGGGTAGTAATTAGATCTGAAAATTTACAGACAATGCTAAAACATAAATAGTACAGCT
GAAGGAATCTGTAGAAATTAAATTGTACAAGACCTAACATAATACAAGAAAAAGTATAACTATAGGACCG
GGGAGAGCATTTTTATGCAACAGGAGACATAATAGGAGATATAAGACAAGCACATTGTAACATTAGTGGAG
AAAAATGGAATAACACTTTTAAAACAGATAGTTACAAAATTACAAGCACAAATTTGGGAATAAAACAATAGT
CTTTAAGCAATCCTCAGGAGGGGACCCAGAAAATTGTAATGCACAGTTTTTAATTGTGGAGGGGAATTTTTTC
TACTGTAATTC AACACAGCTTTTTTAATAGTACTTTGGAATAATACTATAGGGCCAAATAACACTAATGGAA
CTATCACACTCCCATGCAGAATAAAACAAATTATAAACAGGTGGCAGGAAGTAGGAAAAGCAATGTATGC
CCCTCCCATCAGAGGACAAATTAGATGCTCATCAAATATTACAGGACTGCTATTAACAAGAGATGGTGGT
AAAGAGATCAGTAACACCACCGAGATCTTCAGACCTGGAGGTGGAGATATGAGGGACAATTGGAGAAGTG
AATTATATAAATATAAAGTAGTAAAAATTGAGCCATTAGGAGTAGCACCCACCAAGGCAAAGAGAAGAGT
GGTGCAGAGAGAAAAAAGAGCAGTGACGCTAGGAGCTATGTTCCCTTGGGTTCTTGGGAGCAGCAGGAAGC
ACTATGGGCGCACGGTCACTGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAACAGC
AGAACAATTTGCTGAGAGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCA
GCTCCAGGCAAGAGTCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGGGATTTGGGGTTGC
TCTGGAAAACCTATTTGCACCACCTGCTGTGCCTTGGAATGCTAGTTGGAGTAATAAATCTCTGGATCAGA
TTTGGAAATAACATGACCTGGATGGAGTGGGAGAGAGAAAATTGACAATTACACAACTTAATATACACCTT
AATTGAAGAATCGCAGAACCAACAAGAAAAGAAATGAACAAGAATTATTAGAATTGGATAAGTGGGCAAGT
TTGTGGAATTGGTTTGACATATCAAAATGGCTGTGGTATATAAAAAATATTCATAATGATAGTAGGAGGTT
TAGTAGGTTTAAGGATAGTTTTTACTGTGCTTTCTATAGTGAATAGAGTTAGGCAGGGATACTCACCATT
ATCATTTTCAGACCCGCTTCCAGCCCCAAGGGGACCCGACAGGCCCGAAGGAATCGAAGAAGAAGGTGGA
GAGAGAGACAGAGACAGATCCAGTCCATTAGTGCATGGATTATTAGCACTCATCTGGGACGATCTACGGA
GCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTAATCTTGATTGCAGCGAGGATTGTGGAACCTTCT
GGGACGCAAGGGGTGGGAAGCCCTCAAGTATTGGGGGAATCTCCTGCAGTATTGGATTACAGGAATAAAG
AATAGTGCTGTTAGTTTGTGTTGATGCCATAGCTATAGCAGTAGCTGAGGGGACAGATAGGATTATAGAAG
TAGCACAAAGAATTGGTAGAGCTTTTCTCCACATACCTAGAAGAATAAGACAGGGCTTTGAAAGGGCTTT
GCTATAA

FIG. 18 (SEQ ID NO:32)

gp120.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgccctgttctacaagctg
gacgtggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accaggcctgcccgaaggtgagcttcgagcccatcccatccactactgccccccgcccggcttc
gccatcctgaagtgcaacgacaagaagttcaacggcagcggccccctgcaccaacgtgagcaccgtg
cagtgcaccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgag
gagggcgtggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcacccgcccccaacaacaacacccgcaagagcatcaccatcggcccc
ggccgcgccttctacgccaccggcgacatcatcggcgacatccgccaggcccactgcaacatcagc
ggcgagaagtggaacaacacctgaagcagatcgtgaccaagctgcaggcccagttcggcaacaag
accatcgtgttcaagcagagcagcggcgggcgacccccgagatcgtgatgcacagcttcaactgcggc
ggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacaccatcggcccc
aacaacaccaacggcaccatcacctgcccctgccgcatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcggccagatccgctgcagcagcaacatcacccggc
ctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgcgagcagctgtacaagtacaagggtggtgaagatcgagcccctg
ggcgtggccccccaccaaggccaagcgccgcgtggtgcagcgcgagaagcgctaactcgag

FIG. 19 (SEQ ID NO:33)

gp120.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgaccctgcaactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgggcgccggcaagctgatcaactgcaacaccagcgtgatcaccaggcctgcccc
aaggtgagcttcgagcccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagtccaacggcagcggccccctgcaccaacgtgagcaccgtgcagtgcaccacggc
atccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgaggagggcggtggtgatc
cgcagcgagaacttcaccgacaacgccaaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcacccgcccccaacaacaacacccgcaagagcatcaccatcggccccggccgcgccttctac
gccaccggcgacatcatcggcgacatccgccaggccccactgcaacatcagcggcgagaagtggaac
aacaccctgaagcagatcgtgaccaagctgcaggccagttcggcaacaagaccatcgtgttcaag
cagagcagcggcggcgacccccgagatcgtgatgcacagcttcaactgcggcggcgagttcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacaccatcggcccccaacaacaccaacggc
accatcaccttgccttgcgcgatcaagcagatcatcaaccgctggcaggaggtgggcaaggccatg
tacgccccccccatccgcggccagatccgctgcagcagcaacatcacccggcctgctgctgacccgc
gacggcggaaggagatcagcaacaccaccgagatcttccgccccggcgggcgacatgcgcgac
aactggcgcgagcagctgtacaagtacaagtggtgaagatcgagccctgggctggccccacc
aaggccaagcgccgctggtgcagcgcgagaagcgctaactcgag

FIG. 20 (SEQ ID NO:34)

gp120.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggccaaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctggtggcgccggcaactgccagacc
agcgtgatcaccaggcctgcccccaaggtgagcttcgagcccatcccatccactactgcgcccc
gccggcttcgcatcctgaagtgaacgacaagaagttcaacggcagcgccccctgcaccaacgtg
agcaccgtgcagtgcaccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagc
ctggccgaggagggcggtgatccgcagcgagaacttcaccgacaacgccaaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcaccgcgcccccaacaacaacacccgcaagagcatcacc
atcgcccccgccgcgcccttctacgccaccggcgacatcatcgggcgacatccgcccaggcccactgc
aacatcagcggcgagaagtggaacaacaccctgaagcagatcgtgaccaagctgcaggcccagttc
ggcaacaagaccatcgtgttcaagcagagcagcgggcgggcgaccccgagatcgtgatgcacagcttc
aactgcggcgggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgcccccaacaacaccaacggcaccatcacctgcccctgccgcatcaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgcccccccatccgcgccagatccgctgcagcagcaac
atcacggcctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttccgc
ccggcgggcgggcgacatgcgcgacaactggcgcgagcagctgtacaagtacaaggtggtgaagatc
gagccctgggctggccccccaccaaggccaagcgccgcgtggtgcagcgcgagaagcgctaactc
gag

FIG. 21 (SEQ ID NO:35)

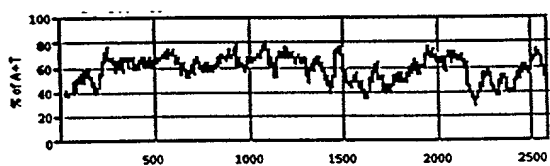


FIG.22A

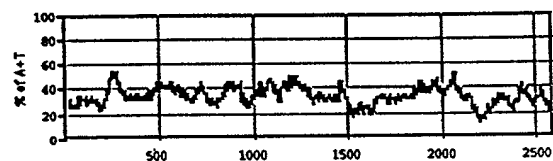


FIG.22B

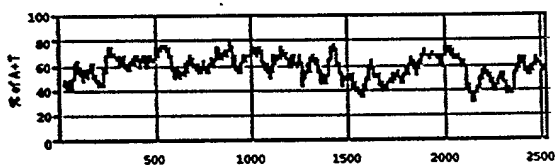


FIG.22E

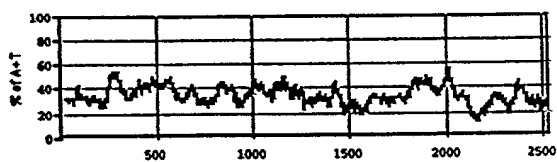


FIG.22F

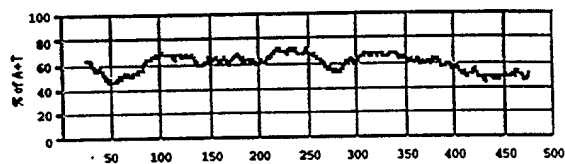


FIG.22C

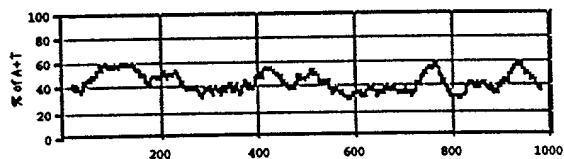


FIG.22D

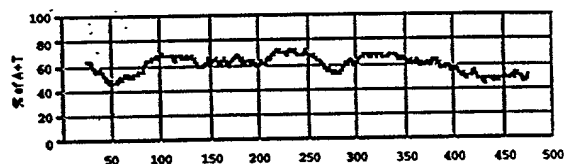


FIG.22G



FIG.22H

gp140.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgcggtgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgcggtgaagctgacccccctgtgctgacccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaagggtgaccaccagcatccgcaacaagatgcagaaggagtacgccctgttctacaagctg
gacgtggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accaggcctgcccgaaggtagcttcgagcccatcccatccactactgccccccgcccggcttc
gccatcctgaagtgcaacgacaagaagttcaacggcagcggccccctgcaccaacgtgagcaccgtg
cagtgcaccacacggcatccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgag
gagggcggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcacccgcccccaacaacacccgcaagagcatcaccatcggtccc
ggccgcgccttctacgccaccggcgacatcatcggcgacatccgccaggccccactgcaacatcagc
ggcgagaagtggaacaacaccctgaagcagatcgtgaccaagctgcaggccccagttcggcaacaag
accatcgtgttcaagcagagcagcggcgggcgacccccgagatcgtgatgcacagcttcaactgcggc
ggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacaccatcggtccc
aacaacaccaacggcaccatcacctgccccgcatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcggccagatccgctgcagcagcaacatcacccgc
ctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgagcagctgtacaagtacaagggtggtgaagatcgagccccctg
ggcggtggccccccaccaaggccaagcgccgctggtgcagcgcgagaagcgccgctgaccctgggc
gccatgttcttgggttccctgggcgcgcggcagcaccatgggcgccccgagcctgaccctgacc
gtgcaggccccgacgtgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgag
gccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggccccgctgctggcc
gtggagcgtacctgaaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgc
accaccgcccgtgccctggaacgccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggccagcctgtgg
aactgggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 23 (SEQ ID NO:36)

gp140.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctgaagctgacccccctgtgctgaccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtggggcgccggcaagctgatcaactgcaacaccagcgtgatcaccaggcctgcccc
aaggtgagcttcgagcccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagttcaacggcagcggccccctgcaccaacgtgagcaccgtgagtgacccacggc
atccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgaggagggcgctgggtgatc
cgagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcacccgcccccaacaacaacaccccgcaagagcatcaccatcgggccccggccgcgcttctac
gccaccggcgacatcatcggcgacatccgccaggcccactgcaacatcagcggcgagaagtggaac
aacaccctgaagcagatcgtgaccaagctgcaggcccagttcggcaacaagaccatcgtgttcaag
cagagcagcggcgccgaccccgagatcgtgatgcacagcttcaactgcggcgccgagttcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacaccatcgggcccaacaacaccaacggc
accatcaccctgccctgccgcatcaagcagatcatcaaccgctggcaggaggtgggcaaggccatg
tacgccccccccatccgcgggccagatccgctgcagcagcaacatcaccggcctgctgctgacccgc
gacggcggaaggagatcagcaacaccaccgagatcttcgccccggcgccggcgacatgcgcgac
aactggcgagcagctgtacaagtacaaggtggtgaagatcgagccctgggcgtggccccacc
aaggccaagcgccgctggtgcagcgcgagaagcgcgccgtgaccctgggcgcatgttcttgggc
ttcttgggcgcccggcagcaccatgggcgcccgcagcctgaccctgaccgtgcaggccccgccag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcccacatcgaggcccagcagcacctg
ctgcagctgaccgtgtggggcatcaagcagctgcaggccccgctgctggccgtggagcgctacctg
aaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgcaccaccgcccgtgcc
tggaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacaccctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggccagcctgtggaactggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 24 (SEQ ID NO:37)

gp140.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctggtggcgccggcaactgccagacc
agcgtgatcaccagggcctgccccaaaggtgagcttcgagcccatccccatccactactgcgcccc
gcccgttcgccatcctgaagtgaacgacaagaagttcaacggcagcgccccctgcaccaacgtg
agcaccgtgcagtgcaccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagc
ctggccgaggagggcgtggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcaccgcgcccaacaacaacacccgcaagagcatcacc
atcgggccccggccgccttctacgccaccggcgacatcatcgggcgacatccgcccaggcccactgc
aacatcagcggcgagaagtggaaacaacacctgaagcagatcgtgaccaagctgcaggcccagttc
ggcaacaagaccatcgtgttcaagcagagcagcggcgccgacccccgagatcgtgatgcacagcttc
aactgcggcgccgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgggcccaacaacaccaacggcaccatcacctgccctgccgcatcaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgcccccccatccgcgccagatccgctgcagcagcaac
atcacggcctgctgctgaccgcgcagggcggaaggagatcagcaacaccaccgagatcttccgc
cccggcgccggcgacatgcgcgacaactggcgcgagcagctgtacaagtacaaggtggtgaagatc
gagccccctgggcgtggccccccaccaaggccaagcgccgcgtggtgcagcgcgagaagcgccgctg
accctgggcgccatgttcctgggcttcctgggcgcgcggcagcaccatgggcgcccgcagcctg
accctgaccgtgcaggcccgcagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccgc
gtgctggccgtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggcaag
ctgatctgcaccaccgcccgtgccctggaacgccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggccc
agcctgtggaactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 25 (SEQ ID NO:38)

gp140.mut.modSF162

gaattcgccaccatggatgcaatgaagagaggggctctgctgtgtgctgctgctgtgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggcaccaccaccctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggcaccacgcctgcggtgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgcggtgaagctgacccccctgtgctgaccctgcactgcaccaacctg
aagaacgcccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtagcgcctgttctacaagctg
gacgtggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accagggcctgccccaaaggtgagcttcgagcccatcccatccactactgcgcccccgccggcttc
gccatcctgaagtgcacgacaagaagttcaacggcagcgccccctgcaccaacgtgagcaccgtg
cagtgcaccacgcatccgccccgtggtgagcaccacagctgctgctgaacggcagcctggccgag
gagggcgtggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcaccgcgccccaaacaacacccgcaagagcatcaccatcgggccc
ggcgcgccttctacgccaccggcgacatcatcggcgacatccgcccaggcccactgcaacatcagc
ggcgagaagtgaacaacaccctgaagcagatcgtgaccaagctgcaggcccagttcggcaacaag
accatcgtgttcaagcagagcagcgggcgacccccgagatcgtgatgcacagcttcaactgcggc
ggcgagtcttctactgcaacagcaccacagctgttcaacagcacctggaacaacaccatcgggccc
aacaacaccaacggcaccatcacctgccccgcatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcgggccagatccgctgcagcagcaacatcacggc
ctgctgctgaccgcgacggcggaaggagatcagcaacaccaccgagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgagcagctgtacaagtacaaggtggtgaagatcgagccccctg
ggcgtggccccccaccaaggccaagcgccgctggtgcagcgcgagaagagcgccgtgaccctgggc
gccatgttcctgggcttcctgggcgcccggcagcaccatgggcgcccgcagcctgaccctgacc
gtgcaggccccgcagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgag
gcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggccccgctgctggcc
gtggagcgtacctgaaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgc
accaccgcccgtgccctggaacgcccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggccagcctgtgg
aactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 26 (SEQ ID NO:39)

gp140.mut.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggccaccacgcctgcgtgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgcgtgaagctgacccccctgtgcgtgaccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgggcgcggcaagctgatcaactgcaacaccagcgtgatcaccaggcctgcccc
aaggtgagcttcgagccccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagttcaacggcagcggccccctgcaccaacgtgagcaccgtgcagtgcaccacggc
atccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgaggaggcggtggtgatc
cgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcaccgcgccccacaacaacacccgcaagagcatcaccatcgcccccgccgcgccttctac
gccaccggcgacatcatcggcgacatccgccaggcccactgcaacatcagcggcgagaagtggaac
aacaccctgaagcagatcgtgaccaagctgcaggcccagttcggcaacaagaccatcgtgttcaag
cagagcagcggcgccgacccccgagatcgtgatgcacagcttcaactgcggcgccgagttcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccccaacaacaccaacggc
accatcacctgccctgccgcatcaagcagatcatcaaccgctggcaggaggtgggcaaggccatg
tacgccccccccatccgcggccagatccgctgcagcagcaacatcacccggcctgctgctgaccgc
gacggcggaaggagatcagcaacaccaccgagatcttcgccccggcgggcgacatgcgcgac
aactggcgagcagctgtacaagtacaagtggtgaagatcgagccctgggcgtggccccacc
aaggccaagcgccgcgtggtgcagcgcgagaagagcgccgtgaccctgggcgcatgttctgggc
ttcctgggcgcgcggcagcaccatgggcgcccgcagcctgaccctgaccgtgcaggcccgcag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccagcagcacctg
ctgcagctgaccgtgtggggcatcaagcagctgcaggcccgcgtgctggcgtggagcgctacctg
aaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgcaccaccgcctgccc
tggaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtggaactgggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 27 (SEQ ID NO:40)

gp140.mut.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggcccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctggtggcgccggcaactgccagacc
agcgtgatcaccagggcctgcccccaaggtgagcttcgagcccatcccatccactactgcgcccc
gccggcttcgccatcctgaagtgaacgacaagaagttcaacggcagcgccccctgcaccaacgtg
agcaccgtgcagtgcaccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagc
ctggccgaggagggcgctggtgatccgcagcgagaacttcaccgacaacgccaaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcaccgcgcccaacaacaacacccgcaagagcatcacc
atcgcccccgccgcgcccttctacgccaccggcgacatcatcgggcgacatccgccaggcccactgc
aacatcagcggcgagaagtggaacaacaccctgaagcagatcgtgaccaagctgcaggcccagttc
ggcaacaagaccatcgtgttcaagcagagcagcgggcgaccccgagatcgtgatgcacagcttc
aactgcggcgggcagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgcccccaacaacaccaacggcaccatcaccctgcctgcccgcacatcaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgcccccccatccgcgccagatccgctgcagcagcaac
atcaccggcctgctgctgaccgcgacggcggaaggagatcagcaacaccaccgagatcttccgc
cccggcgggcgacatgcgcgacaactggcgcgagcagctgtacaagtacaaggtggtgaagatc
gagccccctggcggtggccccccaccaaggccaagcgccgctggtgcagcgcgagaagagcgccgtg
accctgggcgccatgttcctgggcttctgggcgcccggcagcaccatgggcgcccgcagcctg
accctgaccgtgcaggccccgcagctgctgagcgcatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccgc
gtgctggccgtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggcaag
ctgatctgcaccaccgcccgtgccctggaacgccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcc
agcctgtggaactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 28 (SEQ ID NO:41)

gp140.mut7.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggccacccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgacccctgcaactgcaccaacctg
aagaacgcccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgcccctgttctacaagctg
gacgtgggtgcccatcgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accagggcctgccccaaaggtgagcttcgagcccacccccatccactactgcgcccccgccggcttc
gccatcctgaagtgcaacgacaagaagtccaacggcagcggccccctgcaccaacgtgagcacctgtg
cagtgcacccacggcatccgccccgtggtgagcacccagctgctgctgaacggcagcctggccgag
gagggcgtggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcacccgcccccaacaacaacacccgcaagagcatcaccatcgcccc
ggcgcgccttctacgccaccggcgacatcatcggcgacatccgccaggcccactgcaacatcagc
ggcgagaagtggacaacaccctgaagcagatcgtgaccaagctgcaggcccagttcgggaacaag
accatcgtgttcaagcagagcagcggcgggcgacccccgagatcgtgatgcacagcttcaactgcggc
ggcgagttcttctactgcaacagcacccagctgttcaacagcacctggaacaacaccatcgcccc
aacaacaccaacggcaccatcacctgccttgcgcgcatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgcccccccatccgcggccagatccgctgcagcagcaacatcacccggc
ctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgcgagcagctgtacaagtacaagggtggtgaagatcgagcccctg
ggcgtggccccaccaaggccatcagcagcgtggtgcagagcgagaagagcgccgtgaccctgggc
gccatgttcctgggcttcctgggcgcgcgcgcgcagcaccatgggcgcccgcagcctgaccctgacc
gtgcagggcccgccagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgag
gccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccgcgtgctggcc
gtggagcgtacctgaaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgc
accaccgccgtgccctggaacgccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggccagcctgtgg
aactgggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 29 (SEQ ID NO:42)

gp140.mut7.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttgcgccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggccaccaccaccctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggccaccacgcctgcgtgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgcgtgaagctgacccccctgtgcgtgaccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgggcgccggcaagctgatcaactgcaacaccagcgtgatcaccaggcctgcccc
aaggtgagcttcgagcccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagtccaacggcagcgccccctgcaccaacgtgagcaccgtgcagtgcaccacggc
atccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgaggagggcgctggtgatc
cgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcaccgcgcccaacaacaacaccgcgaagagcatcaccatcgcccccgccgcgccttctac
gccaccggcgacatcatcggcgacatccgcccaggcccactgcaacatcagcggcgagaagtggaac
aacaccctgaagcagatcgtgaccaagctgcaggcccagttcggcaacaagaccatcgtgttcaag
cagagcagcgggcgccgacccccgagatcgtgatgcacagcttcaactgcggcgccgagttcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccccaacaacaccaacggc
accatcaccttgccctgcccgcacatcaagcagatcatcaaccgctggcaggaggtgggcaaggccatg
tacgccccccccatccgcgccagatccgctgcagcagcaacatcacccggcctgctgctgacccgc
gacggcggaaggagatcagcaacaccaccgagatcttccgccccggcgggcgacatgcgcgac
aactggcgagcagctgtacaagtacaagtggtgaagatcgagcccctgggctggccccacc
aaggccatcagcagcgtggtgcagagcgagaagagcgccgtgaccctgggcgccatgttccctgggc
ttcctgggcgccggcgagcaccatgggcgcccgagcctgaccctgaccgtgcaggcccgccag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccagcagcacctg
ctgcagctgaccgtgtggggcatcaagcagctgcaggcccgcgtgctggccgtggagcgctacctg
aaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgcaccaccgcccgtgcc
tggaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacaccctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtggaactgggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 30 (SEQ ID NO:43)

gp140.mut7.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gagggcaccaccacctgttctgcccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggccacccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgtggcgccggcaactgccagacc
agcgtgatcaccagggcctgccccaaaggtgagcttcgagcccatcccatccactactgcgcccc
gccggcttcgccatcctgaagtgcacgacaagaagttcaacggcagcggccccctgcaccaacgtg
agcaccgtgcagtgcaccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagc
ctggccgaggagggcgctggtgatccgcagcgcagaacttcaccgacaacgccaaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcaccgcccccaacaacaacacccgcaagagcatcacc
atcgcccccgccgcgcttctacgccaccggcgacatcatcggcgacatccgccaggccccactgc
aacatcagcggcgagaagtgaacaacacctgaagcagatcgtgaccaagctgcaggccccagttc
ggcaacaagaccatcgtgttcaagcagagcagcggcgccgagaccccgagatcgtgatgcacagcttc
aactgcggcgggcaggttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgcccccaacaacaccaacggcaccatcacctgcctgcccgcacatcaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacccccccccatccgcggccagatccgctgcagcagcaac
atcacccgcctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttccgc
cccgccggcgccgacatgcgcgacaactggcgcgagcagctgtacaagtacaaggtggtgaagatc
gagccccctggcgctggcccccaaccaaggccatcagcagcgtggtgcagagcgagaagagcgccgtg
accctgggcgccatgttccctgggcttccctgggcgcccgccggcagcaccatgggcgcccgagcctg
accctgaccgtgcaggccccgcccagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggccccgc
gtgctggccgtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggcaag
ctgatctgcaccaccgcccgtgccctggaacgccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcc
agcctgtggaactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 31 (SEQ ID NO:44)

gp140.mut8.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgaccctgcactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgccctgttctacaagctg
gacgtggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accaggcctgcccgaaggtgagcttcgagcccatcccatccactactgcgcccccgccggcttc
gccatcctgaagtgcaacgacaagaagttcaacggcagcggccccctgcaccaacgtgagcaccgtg
cagtgcaccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgag
gagggcgtggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcaccgcccccaacaacaacaccgcgaagagcatcaccatcgcccc
ggccgcgccttctacgccaccggcgacatcatcggcgacatccgccaggccccactgcaacatcagc
ggcgagaagtggaacaacaccctgaagcagatcgtgaccaagctgcaggccccagttcggcaacaag
accatcgtgttcaagcagagcagcggcgggcgacccccgagatcgtgatgcacagcttcaactgcggc
ggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccc
aacaacaccaacggcaccatcacctgccctgccgcatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgcccccccatccgcggccagatccgctgcagcagcaacatcacccgc
ctgctgctgaccgcgacggcggaaggagatcagcaacaccaccgagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgagcgagctgtacaagtacaagggtggtgaagatcgagccctg
ggcgtggccccaccatcgccatcagcagcgtggtgcagagcgagaagagcgccgtgacctgggc
gccatgttcctgggcttcctgggcgcgcggcgagcaccatgggcgcccgcagcctgacctgacc
gtgcaggccccgcagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgag
gcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggccccgcgtgctggcc
gtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgc
accaccgcgtgccctggaacgccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtgg
aactggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 32 (SEQ ID NO:45)

650621 " 5752450

gp140.mut8.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggccacccacgcctgcgtgccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgcgtgaagctgacccccctgtgcgtgaccctgcactgcaccaacctg
aagaacgcccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtggcgccggcaagctgatcaactgcaacaccagcgtgatcaccaggcctgcccc
aaggtgagcttcgagcccatcccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagttcaacggcagcggccccctgcaccaacgtgagcacccgtgcagtgcaccacggc
atccgccccgtggtgagcacccagctgctgctgaacggcagcctggccgaggaggcgctggtgatc
cgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcacccgcccccaacaacaacacccgcaagagcatcaccatcgccccggcgcgcccttctac
gccaccggcgacatcatcggcgacatccgccaggccactgcaacatcagcggcgagaagtggaac
aacaccctgaagcagatcgtgaccaagctgcaggcccagttcggcaacaagaccatcgtgttcaag
cagagcagcggcgggcgacccccgagatcgtgatgcacagcttcaactgcggcgggcgagttcttctac
tgcaacagcacccagctgttcaacagcacctggaacaacaccatcgcccccaacaacaccaacggc
accatcacccctgccctgccgcatcaagcagatcatcaaccgctggcaggaggtgggcaaggccatg
tacgcccccccatccgcggccagatccgctgcagcagcaacatcacccggcctgctgctgacccgc
gacggcgggcaaggagatcagcaacaccaccgagatcttcgccccggcgggcgacatgcgcgac
aactggcgcgagcagctgtacaagtacaaggtggtgaagatcgagcccctgggcgtggccccacc
atcgccatcagcagcgtggtgcagagcgagaagagcgccgtgaccctgggcgccatgttcttgggc
ttcctgggcgcgcggcgagcaccatgggcgcccgcagcctgaccctgaccgtgcaggcccgcag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccagcagcacctg
ctgcagctgaccgtgtggggcatcaagcagctgcaggcccgcgtgctggcgtggagcgctacctg
aaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgcaccaccgcccgtgcc
tggaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtggaactggttcgacatc
agcaagtggctgtggtacatctaactcgag

FIG. 33 (SEQ ID NO:46)

gp140.mut8.modSF162.delV1V2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccaccctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggccacccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatgggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctggtggcgccggcaactgccagacc
agcgtgatcaccagggcctgcccgaaggtgagcttcgagcccatcccatccactactgcgcccc
gccggcttcgccatcctgaagtgaacgacaagaagtccaacggcagcgggccctgcaccaacgtg
agcaccgtgcagtgcacccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagc
ctggccgaggagggcgctggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtg
cagctgaaggagagcgtggagatcaactgcacccgcccccaacaacaacaccgcgaagagcatcacc
atcgcccccgccgcgcttctacgccaccggcgacatcatcggcgacatccgccaggccactgc
aacatcagcggcgagaagtggaacaacaccctgaagcagatcgtgaccaagctgcaggcccagttc
ggcaacaagaccatcgtgttcaagcagagcagcgggcgaccccgagatcgtgatgcacagcttc
aactgcggcggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacacc
atcgcccccaacaacaccaacggcaccatcaccctgccctgccgcacatcaagcagatcatcaaccgc
tggcaggaggtgggcaaggccatgtacgcccccccatccgcggccagatccgctgcagcagcaac
atcaccggcctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttcgc
cccgcgggcggcgacatgcgcgacaactggcgagcagctgtacaagtacaaggtggtgaagatc
gagcccctgggctggccccaccatcgccatcagcagcgtggtgcagagcgagaagagcgccgtg
accctgggcgccatgttcctgggcttcttgggcgccgcccggcagcaccatgggcgcccgagcctg
accctgaccgtgcaggcccgcagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgc
gccatcgaggcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccgc
gtgctggccgtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggcaag
ctgatctgcaccaccgcccgtgccctggaacgccagctggagcaacaagagcctggaccagatctgg
aacaacatgacctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctg
atcgaggagagccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcc
agcctgtggaactgggttcgacatcagcaagtggctgtggtacatctaactcgag

FIG. 34 (SEQ ID NO:47)

gp160.modSF162

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtgtggagcagtc
ttcgttttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccacctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tggggcaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgaccctgcaactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgaccaccagcatccgcaacaagatgcagaaggagtacgcccgttctacaagctg
gacgtggtgcccacgacaacgacaacaccagctacaagctgatcaactgcaacaccagcgtgatc
accaggcctgcccgaaggtgagcttcgagcccacccccatccactactgccccccgcccgttc
gcatcctgaagtgcaacgacaagaagttaacggcagcggccccctgcaccaacgtgagcacctg
cagtgaccccacggcatccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgag
gagggcgtggtgatccgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaag
gagagcgtggagatcaactgcaccgcccccaacaacacccgcaagagcatcacatcgcccc
ggcgcgccttctacgccaccggcgacatcatcggcgacatccgccaggcccactgcaacatcagc
ggcgagaagtggaacaacacctgaagcagatcgtgaccaagctgcaggcccagttcggcaacaag
accatcgtgttcaagcagagcagcggcgggcgacccccgagatcgtgatgcacagcttcaactgcggc
ggcgagttcttctactgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccc
aacaacaccaacggcaccatcacctgccctgccgcatcaagcagatcatcaaccgctggcaggag
gtgggcaaggccatgtacgccccccccatccgcggccagatccgctgcagcagcaacatcacccggc
ctgctgctgacccgcgacggcggaaggagatcagcaacaccaccgagatcttccgccccggcggc
ggcgacatgcgcgacaactggcgagcgagctgtacaagtacaagggtggtgaagatcgagcccctg
ggcgtggccccccaccaaggccaagcgccgctggtgcagcgcgagaagcgcgccgtgaccctgggc
gccatgttcttgggttcttgggcgcgcggcgagcaccatgggcgcccgcagcctgaccctgacc
gtgcaggcccgcagctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgag
gcccagcagcacctgctgcagctgaccgtgtggggcatcaagcagctgcaggcccgcgtgctggcc
gtggagcgctacctgaaggaccagcagctgctgggcatctggggctgcagcggcaagctgatctgc
accaccgcccgtgccctggaacgccagctggagcaacaagagcctggaccagatctggaacaacatg
acctggatggagtgggagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggag
agccagaaccagcaggagaagaacgagcaggagctgctggagctggacaagtgggcccagcctgtgg
aactggttcgacatcagcaagtggctgtggtacatcaagatcttcatcatgatcgtgggcggcctg
gtgggcctgcgcacgtgttccacgtgctgagcatcgtgaaccgctgcgccagggtacagcccc
ctgagcttcagaccccgttccccgccccccgcgggccccgaccgccccgagggcatcgaggaggag
ggcggcgagcgcgaccgcgaccgcagcagccccctggtgcacggcctgctggccctgatctgggac
gacctgcgcagcctgtgcctgttcagctaccaccgctgcgcgacctgatcctgatcgccgccccgc
atcgtggagctgctgggcccgcgcggctgggaggccctgaagtactggggcaacctgctgcagtac
tggatccaggagctgaagaacagcgccgtgagcctgttcgacgccatcgccatcgccgtggccgag
ggcaccgaccgcatcatcgaggtggcccagcgcatcgccgcgccttcttgcacatccccgcgcgc
atccgcccagggttcgagcgcgccctgctgtaactcgag

FIG. 35 (SEQ ID NO:48)

gp160.modSF162.delV2

gaattcgccaccatggatgcaatgaagagagggctctgctgtgtgctgctgctgtgtggagcagtc
ttcgtttcgcccagcgccgtggagaagctgtgggtgaccgtgtactacggcgtgcccgtgtggaag
gaggccaccaccaccctgttctgcgccagcgacgccaaggcctacgacaccgaggtgcacaacgtg
tgggccaccacgcctgctgcccaccgaccccaacccccaggagatcgtgctggagaacgtgacc
gagaacttcaacatgtggaagaacaacatggtggagcagatgcacgaggacatcatcagcctgtgg
gaccagagcctgaagccctgctggaagctgacccccctgtgctgaccctgactgcaccaacctg
aagaacgccaccaacaccaagagcagcaactggaaggagatggaccgcggcgagatcaagaactgc
agcttcaaggtgggcgccggcaagctgatcaactgcaacaccagcgtgatcaccaggcctgcccc
aaggtgagcttcgagcccatccccatccactactgcgcccccgccggcttcgccatcctgaagtgc
aacgacaagaagttcaacggcagcggccccctgcaccaacgtgagcaccgtgcagtgcaccacggc
atccgccccgtggtgagcaccagctgctgctgaacggcagcctggccgaggagggcggtgatc
cgcagcgagaacttcaccgacaacgccaagaccatcatcgtgcagctgaaggagagcgtggagatc
aactgcaccgcgcccccaacaacaacacccgcaagagcatcaccatcgccccggcgcgcccttctac
gccaccggcgacatcatcggcgacatccgcccaggcccactgcaacatcagcggcgagaagtggaac
aacaccctgaagcagatcgtgaccaagctgcaggcccagttcggaacaagaccatcgtgttcaag
cagagcagcggcgggcgaccccgagatcgtgatgcacagcttcaactgcggcgggcgagttcttctac
tgcaacagcaccagctgttcaacagcacctggaacaacaccatcgcccccaacaacaccaacggc
accatcaccttgccctgcccgcacatcaagcagatcatcaaccgctggcaggaggtgggcaaggccatg
tacgccccccccatccgcgggccagatccgctgcagcagcaacatcacccggcctgctgctgaccgc
gacggcggaaggagatcagcaacaccaccgagatcttcgccccggcgggcgacatgcgcgac
aactggcgagcagctgtacaagtacaagtggtgaagatcgagcccctggcgctggccccacc
aaggccaagcgccgctggtgcagcgcgagaagcgcgccgtgaccctggcgccatgttcctgggc
ttcctgggcgcccgcggcagcaccatgggcgcccgcagcctgaccctgaccgtgcaggcccgcag
ctgctgagcggcatcgtgcagcagcagaacaacctgctgcgcgccatcgaggcccagcagcacctg
ctgcagctgaccgtgtggggcatcaagcagctgcaggcccgcgtgctggccgtggagcgctacctg
aaggaccagcagctgctggggcatctggggctgcagcggcaagctgatctgcaccaccgcccgtgcc
tggaacgccagctggagcaacaagagcctggaccagatctggaacaacatgacctggatggagtgg
gagcgcgagatcgacaactacaccaacctgatctacacctgatcgaggagagccagaaccagcag
gagaagaacgagcaggagctgctggagctggacaagtgggccagcctgtggaactggttcgacatc
agcaagtggctgtggtacatcaagatcttcatcatgatcgtgggcggcctgggtgggcctgcgcac
gtgttcaccgtgctgagcatcgtgaaccgctgcgccagggctacagccccctgagcttcagacc
cgcttccccgccccccgcccgcacccgccccgagggcatcgaggaggagggcggcgagcgcgac
cgcgaccgcagcagccccctggtgcacggcctgctggccctgatctgggacgacctgcgcagcctg
tgctgttcagctaccaccgctgcgcgacctgatcctgatcgccgcccgcacgtggagctgctg
ggccgcccggctgggaggccctgaagtactggggcaacctgctgcagtactggatccaggagctg
aagaacagcgccgtgagcctgttcgacgccatcgccatcgccgtggccgagggcaccgaccgcac
atcgaggtggcccagcgcacggccgcgcttctgacatcccccgccgcacccgcccagggttc
gagcgcgcccctgctgtaactcag

FIG. 36 (SEQ ID NO:49)

[illegible]

gp160.modSF162.delV1V2

gp160.modSF162.delV1V2

gp120wtUS4

ACAACAGTCTTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAG
CAACCACCACTCTGTTTTGTGCATCAGATGCTAAAGCATACAAAGCAGAGGC
ACATAACGTCTGGGCTACACATGCCTGTGTACCCACAGACCCCAACCCACAG
GAAGTAAATTTAACAAATGTGACAGAAAATTTTAAACATGTGGAAAAATAACA
TGGTGGAAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAA
GCCATGTGTAAAATTAACCCCACTCTGTGTTACTTTAAATTGTACTGATAAGT
TGACAGGTAGTACTAATGGCACAAATAGTACTAGTGGCACTAATAGTACTAG
TGGCACTAATAGTACTAGTACTAATAGTACTGATAGTTGGGAAAAGATGCCA
GAAGGAGAAATAAAAAACTGCTCTTTC AATATCACCACAAGTGTAAGAGATA
AAGTGCAGAAAGAATATTCTCTCTTCTATAAACTTGATGTAGTACCAATAGAT
AATGATAATGCTAGCTATAGATTGATAAATTGTAATACCTCAGTCATTACACA
AGCCTGTCCAAAGGTATCTTTTGAACCAATTCCCATACATTATTGTGCCCCGG
CTGGTTTTTGCGATTCTAAAGTGTAAGATAAGAAGTTCAATGGAACAGGACC
ATGTAAAAATGTCAGCACAGTACAATGCACACATGGAATTAGACCAGTAGTA
TCAACTCAACTGCTGTAAATGGCAGTCTAGCAGAAGAAGAGATAGTACTTA
GATCTGAAAATTTACACAGACAATGCTAAAACCATAATAGTACAGCTGAATGA
ATCTGTAGAAATTAATTGTATAAGACCCAACAATAATAACAAGAAAAAGTATA
CATATAGGACCAGGGAGAGCATTTTATGCAACAGGTGATATAATAGGAGACA
TAAGACAAGCACATTGTAACATTAGTAAAGCAAACCTGGACTAACACTTTAGA
ACAGATAGTTGAAAAATTAAGAGAACAATTTGGGAATAATAAAACAATAATC
TTTAATTCATCCTCAGGAGGGGACCCAGAAATTGTATTTACAGTTTTAATTG
TGGAGGGGAATTTTTCTATTGTAATACATCACAACCTATTTAATAGTACCTGGA
ATATTACTGAAGAGGTAAATAAGACTAAAGAAAATGACACTATCATACTCCC
ATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGGAAAAGCAAT
GTATGCCCCTCCCATCAGAGGACAAATTAAATGTTTCATCAAATATTACAGGG
CTGCTATTAAGTATAGAGATGGTGGTACTAACAATAATAGGACGAACGACACCG
AGACCTTCAGACCTGGGGGAGGAAACATGAAGGACAATTGGAGAAGTGAAT
TATATAAATATAAAGTAGTAAGAATTGAACCATTAGGAGTAGCACCCACCCA
GGCAAAGAGAAGAGTGGTGCAAAGAGAGAAAAGA

FIG. 38 (SEQ ID NO:51)

650221 " 5' 5' 460

gp140wtUS4

ACAACAGTCTTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAG
CAACCACCACTCTGTTTTGTGCATCAGATGCTAAAGCATACAAAGCAGAGGC
ACATAACGTCTGGGCTACACATGCCTGTGTACCCACAGACCCCAACCCACAG
GAAGTAAATTTAACAAATGTGACAGAAAAATTTTAACATGTGGAAAAATAACA
TGGTGGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAA
GCCATGTGTAAAATTAACCCCACTCTGTGTTACTTTAAATTGTACTGATAAGT
TGACAGGTAGTACTAATGGCACAATAAGTACTAGTGGCACTAATAGTACTAG
TGGCACTAATAGTACTAGTACTAATAGTACTGATAGTTGGGAAAAGATGCCA
GAAGGAGAAATAAAAAACTGCTCTTTCAATATCACCACAAGTGTAAGAGATA
AAGTGCAGAAAGAATATTCTCTCTTCTATAAACTTGATGTAGTACCAATAGAT
AATGATAATGCTAGCTATAGATTGATAAATTGTAATACCTCAGTCATTACACA
AGCCTGTCCAAAGGTATCTTTTGAACCAATTCCCATACATTATTGTGCCCCGG
CTGGTTTTGCGATTCTAAAGTGTAAGATAAGAAGTTCAATGGAACAGGACC
ATGTAAAAATGTCAGCACAGTACAATGCACACATGGAATTAGACCAGTAGTA
TCAACTCAACTGCTGTAAATGGCAGTCTAGCAGAAGAAGAGATAGTACTTA
GATCTGAAAATTTACAGACAATGCTAAAACCATAATAGTACAGCTGAATGA
ATCTGTAGAAATTAATTGTATAAGACCCAACAATAACAAGAAAAAGTATA
CATATAGGACCAGGGAGAGCATTTTATGCAACAGGTGATATAATAGGAGACA
TAAGACAAGCACATTGTAACATTAGTAAAGCAAACCTGGACTAACACTTTAGA
ACAGATAGTTGAAAAATTAAGAGAACAATTTGGGAATAATAAAACAATAATC
TTTAATTCATCCTCAGGAGGGGACCCAGAAATTGTATTTACAGTTTTAATTG
TGGAGGGGAATTTTTCTATTGTAATACATCACAACCTATTTAATAGTACCTGGA
ATATTACTGAAGAGGTAAATAAGACTAAAGAAAATGACACTATCATACTCCC
ATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGGAAAAGCAAT
GTATGCCCCTCCCATCAGAGGACAAATTAAATGTTTCATCAAATATTACAGGG
CTGCTATTAAGTACTAGAGATGGTGGTACTAACAATAATAGGACGAACGACACCG
AGACCTTCAGACCTGGGGGAGGAAACATGAAGGACAATTGGAGAAGTGAAT
TATATAAATATAAAGTAGTAAGAATTGAACCATTAGGAGTAGCACCCACCCA
GGCAAAGAGAAGAGTGGTGCAAAGAGAGAAAAGAGCAGTGGGACTAGGAG
CTTTGTTCAATTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTC
AGTGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAACAG
CAGAACAATTTGCTGAGAGCTATTGAGGCGCAACAGCATCTGTTGCAACTCA
CGGTCTGGGGCATCAAACAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA
CCTAAAGGATCAACAGCTCCTAGGGATTTGGGGTTGCTCTGGAAAACCTCATTT
GCACCACTACTGTGCCTTGGAACCTAGTTGGAGTAATAAATCTCTGACTGAG
ATTTGGGATAATATGACCTGGATGGAGTGGGAAAGAGAAATTGGCAATTATA
CAGGCTTAATATACAATTTAATTGAAATAGCACAAAACCAGCAAGAAAAGAA
TGAACAAGAATTATTGGAATTAGACAAGTGGGCAAGTTTGTGGAATTGGTTT
GATATAACAACTGGCTGTGGTATATA

FIG. 39 (SEQ ID NO:52)

gp160wtUS4

ACAACAGTCTTGTGGGTCACAGTCTATTATGGGGTACCTGTGTGGAAAGAAG
CAACCACCACTCTGTTTTGTGCATCAGATGCTAAAGCATACAAAGCAGAGGC
ACATAACGTCTGGGCTACACATGCCTGTGTACCCACAGACCCCAACCCACAG
GAAGTAAATTTAACAAATGTGACAGAAAATTTTAACATGTGGAAAAATAACA
TGGTGGAACAGATGCATGAGGATATAATCAGTTTATGGGATCAAAGCCTAAA
GCCATGTGTAAAATTAACCCCACTCTGTGTTACTTTAAATTGTACTGATAAGT
TGACAGGTAGTACTAATGGCACAAATAGTACTAGTGGCACTAATAGTACTAG
TGGCACTAATAGTACTAGTACTAATAGTACTGATAGTTGGGAAAAGATGCCA
GAAGGAGAAATAAAAAACTGCTCTTTCAATATCACCACAAGTGTAAGAGATA
AAGTGCAGAAAGAATATTCTCTCTTCTATAAACTTGATGTAGTACCAATAGAT
AATGATAATGCTAGCTATAGATTGATAAATTGTAATACCTCAGTCATTACACA
AGCCTGTCCAAAGGTATCTTTTGAACCAATTCCCATACATTATTGTGCCCCGG
CTGGTTTTGCGATTCTAAAGTGTAAGATAAGAAGTTCAATGGAACAGGACC
ATGTAAAAATGTCAGCACAGTACAATGCACACATGGAATTAGACCAGTAGTA
TCAACTCAACTGCTGTAAATGGCAGTCTAGCAGAAGAAGAGATAGTACTTA
GATCTGAAAATTTACACAGACAATGCTAAAACCATAATAGTACAGCTGAATGA
ATCTGTAGAAATTAATTGTATAAGACCCAACAATAACAAGAAAAAGTATA
CATATAGGACCAGGGAGAGCATTTTATGCAACAGGTGATATAATAGGAGACA
TAAGACAAGCACATTGTAACATTAGTAAAGCAAACCTGGACTAACACTTTAGA
ACAGATAGTTGAAAAATTAAGAGAACAATTTGGGAATAATAAAACAATAATC
TTTAATTCATCCTCAGGAGGGGACCCAGAAATTGTATTTACAGTTTTTAATTG
TGGAGGGGAATTTTTCTATTGTAATACATCACAACCTATTTAATAGTACCTGGA
ATATTACTGAAGAGGTAAATAAGACTAAAGAAAATGACACTATCATACTCCC
ATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGGAAAAGCAAT
GTATGCCCCTCCCATCAGAGGACAAATTAATGTTTCATCAAATATTACAGGG
CTGCTATTAAGTATAGAGATGGTGGTACTAACAATAATAGGACGAACGACACCG
AGACCTTCAGACCTGGGGGAGGAAACATGAAGGACAATTGGAGAAGTGAAT
TATATAATATAAAGTAGTAAGAATTGAACCATTAGGAGTAGCACCCACCCA
GGCAAAGAGAAGAGTGGTGCAAAGAGAGAAAAGAGCAGTGGGACTAGGAG
CTTTGTTTCATTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTC
AGTGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGCAACAG
CAGAACAATTTGCTGAGAGCTATTGAGGCGCAACAGCATCTGTTGCAACTCA
CGGTCTGGGGCATCAAACAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA
CCTAAAGGATCAACAGCTCCTAGGGATTTGGGGTTGCTCTGGAAAACCTATTT
GCACCACTACTGTGCCTTGGAACCTAGTTGGAGTAATAAATCTCTGACTGAG
ATTTGGGATAATATGACCTGGATGGAGTGGGAAAGAGAAATTGGCAATTATA
CAGGCTTAATATACAATTTAATTGAAATAGCACAAAACCAGCAAGAAAAGAA
TGAACAAGAATTATTGGAATTAGACAAGTGGGCAAGTTTGTGGAATTGGTTT
GATATAACAACTGGCTGTGGTATATAAGAATATTCATAATGATAGTAGGAG
GCTTGATAGGTTTAAGAATAGTTTTTGTCTGTACTTTCTATAGTGAATAGAGTT
AGGCAGGGATACTACCAATATCATTGCAGACCCGCCTCCCAGCTCAGAGGG

FIG. 40 (SEQ ID NO:53)

GACCCGACAGGCCCGAAGGAATCGAAGAAGAAGGTGGAGAGAGAGACAGA
GACAGATCCAATCGATTAGTGCATGGATTATTGGCACTCATCTGGGACGATCT
GCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTG
TAGCGAGGATTGTGGAACCTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAGTA
TTGGTGGAATCTCCTGCAGTATTGGAGTCAGGAGCTAAAGAGTAGTGCTGTT
AGTTTGTTTAATGCCACAGCAATAGCAGTAGCTGAAGGGACAGATAGGATTA
TAGAAATAGTACAAAGAATTTTTAGAGCTGTAATTCACATACCTAGAAGAAT
AAGACAGGGCTTGGAGAGGGCTTTACTATAA

FIG. 40 CONT'D (SEQ ID NO:53)

gp120.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTGCCCGTG
TGGAAGGAGGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCAGCGGCAC
CAACAGCACCGCGGCACCAACAGCACCAGCACCAACAGCACCAGCTGGGAGAAGATG
CCCGAGGGCGAGATCAAGAAGCTGCAGCTTCAACATCACCACCGCTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGCCCCCTGCAAGAAGCTGAGCACCGTGAGTGACCCACGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTACCGACAACGCCAAGACCATCATCGTGAGCTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCT
ACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCAA
CGACACCGAGACCTTCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGGCCCCCACCAGGCCAAGCGCCGC
GTGGTGACGCGGAGAAGCGCTAAGATATCGGATCCTCTAGA

FIG. 41 (SEQ ID NO:54)

gp120.mod.US4.del128-194

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCCAACAGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGCGGGCAGGGAAGTGCAGAGACCAGCGTGATCACCAGGC
CTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGC
ACCGTGCAAGTGCACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGG
CAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACCGACAACGCCAAGA
CCATCATCGTGCAAGTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAAC
ACGCGTAAGAGCATCCACATCGGCCCCCGCGCGCCTTCTACGCCACCGGCGACATCAT
CGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCCTCG
AGCAGATCGTGGAAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTT
CTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCAGGAGGTGA
ACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAAC
ATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTG
CAGCAGCAATATTACCGGCCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCA
CCAACGACACCGAGACCTTCCGCCCCGGCGGCGCAACATGAAGGACAACCTGGCGCAGC
GAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGGCCCCCACCAGGC
CAAGCGCCGCGTGGTGAGCGCGAGAAGCGCTAAGATATCGGATCCTCTAGA

FIG. 42 (SEQ ID NO:55)

[illegible]

FIG. 43 (SEQ ID NO:56)

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GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCAGCGGCAC
CAACAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACCAGACAGCTGGGAGAAGATG
CCCGAGGGCGAGATCAAGAACTGCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCCAGGCCTGCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGCAAGTGACCCACGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTCACCGACAACGCCAAGACCATCATCGTGACGCTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCCCGCGCGCCTTCT
ACGCCACCGGCAGATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCCGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCAGATCAAGTGC
AGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGCCCCCAACCAGGCCAAGCGCCGC
GTGGTGACGCGGAGAAGAGCGCCGTGGGCCTGGGCGCCCTGTTTCATCGGCTTCCTGGGCGCC
GCCGGGAGCACCATGGGCGCCGCCTCCGTGACCCTGACCGTGACGGCCCGCCAGCTGCTGAG
CGGCATCGTGACGAGCAGAACAACCTGCTGCGCGCCATCGAGGCCCGAGCAGCACCTGCTGC
AGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCGCTACCTG
AAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCACCACCGT
GCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
TGGAGTGGGAGCGGAGATCGGCAACTACCCGGCCTGATCTACAACCTGATCGAGATCGCC
CAGAACCGAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGT
GGAAGTGGTTCGACATCACCAACTGGCTGTGGTACATCTAAGATATCGGATCCTCTAGA

FIG. 44 (SEQ ID NO:57)

gp140.TM.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCAGCGGCAC
CAACAGCACCGCGGCACCAACAGCACCGACCAACAGCACCGACAGCTGGGAGAAGATG
CCCGAGGGCGAGATCAAGAACTGCAGCTTCAACATCACCAACAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCCAGGCCTGCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGCCCCCTGCAAGAACGTGAGCACCGTGACGTGCACCCACGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTCACCGACAACGCCAAGACCATCATCGTGACGTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCGGGCCGCGCCTTCT
ACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCCGGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGCCCCCACCCAGGCCAAGCGCCGC
GTGGTGACGCGGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGTTTCATCGGCTTCTGGGCGCC
GCCGGGAGCACCATGGGCGCCGCTCCGTGACCCCTGACCGTGACGGCCCGCCAGCTGCTGAG
CGGCATCGTGACGAGCAGAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGC
AGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGCTACCTG
AAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCACCACCGT
GCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
TGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCC
CAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGT
GGAACCTGGTTCGACATCACTAAGTGGCTGTGGTACATCCGCATCTTCATCATGATCGTGGGCG
GCCTGATCGGCCCTGCGCATCGTGTTCCGCGTGCTGAGCATCGTGTAAGATATCGGATCCTCTA
GA

FIG. 45 (SEQ ID NO:58)

Gp140modUS4.DV1V2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGC
TGTGTGGAGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACC
GTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCG
CCAGCGACGCCAAGGCTTACAAGGCCGAGGCCACAACGTGTGGGCCACCCA
CGCCTGCGTGCCCAACGACCCCAACCCCCAGGAGGTGAACCTGACCAACGTG
ACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGGGCGCCGGCC
AGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
CGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCCGGC
CCCTGCAAGAACGTGAGCACCGTGCAGTGCACCCACGGCATCCGCCCCGTGG
TGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCT
GCGCTCCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGACGCTGAAC
GAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGTAAGAGCA
TCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCGA
CATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTC
GAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATC
ATCTTCAACAGCAGCAGCGGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCA
ACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCAC
CTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT
CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGGCAAG
GCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAATATTA
CCGGCCTGCTGCTGACCCGCGACGGCGGCCACCAACAACAACCGCACCAACGA
CACCGAGACCTTCCGCCCCGGCGGCCGCAACATGAAGGACAACCTGGCGCAGC
GAGCTGTACAAGTACAAGGTGGTGCATCGAGCCCCCTGGGCGTGGCCCCCA
CCCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAGCGCGCCGTGGGCCTGG
GCGCCCTGTTTCATCGGCTTCCTGGGCGCCGCGGGAGCACCATGGGCGCCGC
CTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCTGAGCGGCATCGTGACG
CAGCAGAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGCAGC
TGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCG
CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTG
ATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGA
CCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCA
ACTACACCGGCCTGATCTACAACCTGATCGAGATCGCCAGAACCAGCAGGA
GAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAA
CTGGTTCGACATCACCAACTGGCTGTGGTACATCTAAGATATCGGATCCTCTA
GA

FIG. 46 (SEQ ID NO:59)

Gp140modUS4.DV2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGC
TGTGTGGAGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACC
GTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCG
CCAGCGACGCCAAGGCTTACAAGGCCGAGGCCACAACGTGTGGGCCACCCA
CGCCTGCGTGCCCAACGACCCCAACCCCAAGGAGGTGAACCTGACCAACGTG
ACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCC
CCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGG
CACCAACAGCACCAGCGGCACCAACAGCACCAGCGGCACCAACAGCACCAG
CACCAACAGCACCAGCAGCTGGGAGAAGATGCCCCGAGGGCGAGATCAAGAA
CTGCAGCTTCAACATCGGCGCCGGCCGCTGATCAACTGCAACACCAGCGTG
ATCACCCAGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACT
GCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGG
CACCGGCCCTGCAAGAACGTGAGCACCCTGCAGTGCACCCACGGCATCCGC
CCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGA
TCGTGCTGCGCTCCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGCA
GCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCAACAACAACACGCGT
AAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCA
TCGGCGACATCCGCCAGGCCACTGCAACATCAGCAAGGCCAACTGGACCAA
CACCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAA
GACCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCAC
AGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAA
CAGCACCTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACAC
CATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTG
GGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCA
ATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCAC
CAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTG
GCGCAGCGAGCTGTACAAGTACAAGGTGGTGCATCGAGCCCCTGGGCGTG
GCCCCACCCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAGCGCGCCGTG
GGCCTGGGCGCCCTGTTTCATCGGCTTCCTGGGCGCCGCGGGAGCACCATGG
GCGCCGCTCCGTGACCCTGACCGTGCAGGCCCCGCCAGCTGCTGAGCGGCAT
CGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTG
CTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCG
TGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGG
CAAGCTGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAG
AGCCTGACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAG
ATCGGCAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCCAGAACC
AGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCC
TGTGGAAGTGGTTCGACATCACCAACTGGCTGTGGTACATCTAAGATATCGG
ATCCTCTAGA

FIG. 47 (SEQ ID NO:60)

Gp140modmutUS4.DV1V2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGC
TGTGTGGAGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACC
GTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCG
CCAGCGACGCCAAGGCTTACAAGGCCGAGGCCACAACGTGTGGGCCACCC
ACGCCTGCGTGCCCAACGACCCCAACCCCCAGGAGGTGAACCTGACCAACGT
GACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGA
GGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGGGCGCGCGGC
CAGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCC
CCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGG
CCCCTGCAAGAACGTGAGCACCGTGCAAGTGCAACCCACGGCATCCGCCCCGTG
GTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGC
TGCCTCCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGCAGCTGAA
CGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAAACACGCGTAAGAGC
ATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCG
ACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCT
CGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCAT
CATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCACAGCTTC
AACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCA
CCTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCA
TCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGGCAA
GGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAATATT
ACCGGCCTGCTGCTGACCCGCGACGGCGGCCACCAACAACAACCGCACCAACG
ACACCGAGACCTTCCGCCCCGGCGGCGGCCAACATGAAGGACAACCTGGCGCA
GCGAGCTGTACAAGTACAAGGTGGTGCATCGAGCCCCTGGGCGTGGCCCC
CACCCAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAGAGCGCCGTGGGCCT
GGGCGCCCTGTTTCATCGGCTTCCCTGGGCGCCCGCGGAGCACCATGGGCGCC
GCCTCCGTGACCCTGACCGTGCAGGCCCGCCAGCTGCTGAGCGGCATCGTGC
AGCAGCAGAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTGCTGCA
GCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAG
CGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGC
TGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCT
GACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGG
CAACTACACCGGCCTGATCTACAACCTGATCGAGATCGCCAGAACCAGCAG
GAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGG
AACTGGTTCGACATCACCAACTGGCTGTGGTACATCTAAGATATCGGATCCTC
TAGA

FIG. 48 (SEQ ID NO:61)

gp140.mod.US4.del128-194

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGACTACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCCAACAGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAATTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGGGGGCAGGGAAGTGCAGAGACCAGCGTGATCACCAGGC
CTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCACGGCACCGGCCCTGCAAGAACGTGAGC
ACCGTGCAGTGCACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGG
CAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAAGTTCACCGACAACGCCAAGA
CCATCATCGTGCACTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAAC
ACGCGTAAGAGCATCCACATCGGCCCCCGCCGCGCCTTCTACGCCACCGGCGACATCAT
CGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCG
AGCAGATCGTGGAAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTT
CTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCAGGAGGTGA
ACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAAC
ATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCCAGATCAAGTG
CAGCAGCAATATTACCGGCCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCA
CCAACGACACCGAGACCTTCCGCCCCGGCGGCGCAACATGAAGGACAACCTGGCGCAGC
GAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGGCCCCCACCAGGC
CAAGCGCCGCGTGGTGAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGTTTCATCG
GCTTCCTGGGCGCCGCCGGGAGCACCATGGGCGCCGCCTCCGTGACCCCTGACCGTGAG
GCCCCGCCAGCTGCTGAGCGGCATCGTGAGCAGCAGACAACCTGCTGCGCGCCATCGA
GGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCA
TCCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGC
GGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCT
GACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACA
CCGGCCTGATCTACAACCTGATCGAGATCGCCAGAACCAGCAGGAGAAGAACGAGCAG
GAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAAGTGGTTCGACATCACCAACTG
GCTGTGGTACATCTAAGATATCGGATCCTCTAGA

FIG. 49 (SEQ ID NO:62)

660627-5152460

gp140.mut.mod.US4.del128-194

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCCAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGGGGGCAGGGAAGTGCAGAGACCAGCGTGATCACCAGGC
CTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAAGCTGAGC
ACCGTGCAGTGCACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGG
CAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACCGACAACGCCAAGA
CCATCATCGTGCACTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAAC
ACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCAT
CGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCG
AGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTT
CTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCAGGAGGTGA
ACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAAC
ATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCCAGATCAAGTG
CAGCAGCAATATTACCGGCCCTGTGCTGACCCGCGACGGCGGCACCAACAACAACCGCA
CCAACGACACCGAGACCTTCCGCCCCGGCGGCGCAACATGAAGGACAACCTGGCGCAGC
GAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGGCCCCCACCAGGC
CAAGCGCCGCGTGGTGAGCGCGAGAAGAGCGCCGTGGGCCTGGGCGCCCTGTTTCATCG
GCTTCCTGGGCGCCGCCGGGAGCACCATGGGCGCCGCCTCCGTGACCCTGACCGTGCAAG
GCCCCGAGCTGCTGAGCGGCATCGTGAGCAGCAGAACCAACCTGCTGCGCGCCATCGA
GGCCCAGCAGCACCTGCTGAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCA
TCCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGC
GGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCT
GACCGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACA
CCGGCCTGATCTACAACCTGATCGAGATCGCCAGAACAGCAGGAGAAGAAGAGCAG
GAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAAGTGGTTCGACATCACCAACTG
GCTGTGGTACATCTAAGATATCGGATCCTCTAGA

FIG. 50 (SEQ ID NO:63)

gp160.modUS4

GAATTCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTGCCCCGTG
TGGAAGGAGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCCAACGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAAATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAAGTGCACCGACAAGCTGACCGGCAGCACCAACGGCACCAACAGCACCAGCGGCAC
CAACAGCACCGCGGCACCAACAGCACCAGCACCAACAGCACCAGCTGGGAGAAGATG
CCCGAGGGCGAGATCAAGAACTGCAGCTTCAACATCACCAACAGCGTGCGCGACAAGGTGCA
GAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACGACAACGCCAGCT
ACCGCCTGATCAACTGCAACACCAGCGTGATCACCCAGGCCTGCCCAAGGTGAGCTTCGAGC
CCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGT
TCAACGGCACCGGCCCCCTGCAAGAACGTGAGCACCGTGCAAGTGACCCACGGCATCCGCCCC
GTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTC
CGAGAACTTCACCGACAACGCCAAGACCATCATCGTGAGCTGAACGAGTCCGTGGAGATCA
ACTGCATCCGCCCCAACAAACACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCT
ACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAAC
TGGACCAACACCCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTTCGGCAACAACAAGAC
CATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGA
GGTGAACAAGACCAAGGAGAACGACACCATCATCTGCCCTGCCGCATCCGCCAGATCATCA
ACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGC
AGCAGCAATATTACCGGCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCA
CGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGT
ACAAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGCCCCCACCCAGGCCAAGCGCCGC
GTGGTGCAAGCGGAGAAGCGCGCCGTGGGCGCTGGGCGCCCTGTTTCATCGGCTTCTGGGCGCC
GCCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACGGCCCGCCAGCTGCTGAG
CGGCATCGTGACGAGCAGACAACCTGCTGCGCGCCATCGAGGCCCGAGCAGCACCTGCTGC
AGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGCTACCTG
AAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGCAAGCTGATCTGCACCACCACCGT
GCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGA
TGGAGTGGGAGCGGAGATCGGCAACTACACCGCCTGATCTACAACCTGATCGAGATCGCC
CAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGT
GGAAGTGGTTCGACATCACCAACTGGCTGTGGTACATCCGCATCTTCATCATGATCGTGGGCG
GCCTGATCGGCCTGCGCATCGTGTTCCGCGTGCTGAGCATCGTGAACCGCGTGCGCCAGGGCT
ACAGCCCCATCAGCCTGCAGACCCGCCTGCCCCCGAGCGCGGCCCGACCGCCCCGAGGGC
ATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCAGCAACCGCCTGGTGCACGGCCTGCT
GGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCTGTTTCAGCTACCACCGCCTGCGCGACCT
GCTGCTGATCGTGGCCCGCATCGTGGAGCTGTGGGCCCGCGGCTGGGAGGCCCTGAAGT
ACTGGTGGAACTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTT
AACGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCAT
CTTCCGCGCCGTGATCCACATCCCCCGCGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTA
AGATATCGGATCCTCTAGA

FIG. 51 (SEQ ID NO:64)

gp160.modUS4.delV1

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTGACCCCCCTGTGCGTG
ACCCTGAACTGCACCGACAAGCTGGGCGCCGGCGGCGAGATCAAGAACTGCAGCTTCAACAT
CACCACCAGCGTGCGCGACAAGGTGCAGAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGG
TGCCCATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCAACACCAGCGTGATCACCC
AGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCG
CCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCCGGCCCCCTGCAAGAACGTGAGCACC
GTGCAGTGCACCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTG
GCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACCGACAACGCCAAGACCATCATCGT
GCAGCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGTAAGAGCA
TCCACATCGGCCCCCGCCGCTTCTACGCCACCGGCGACATCATCGGCGACATCCGCCAGG
CCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAGATCGTGGGAGAAGCTG
CGCGAGCAGTTTCGGCAACAACAAGACCATCATCTTCAACAGCAGCAGCGGCGGCGACCCGA
GATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTT
CAACAGCACCTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCATCC
TGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCC
CCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGACCCGCGAC
GGCGGCACCAACAACAACCGCACCAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACAT
GAAGGACAACCTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCG
TGCGCCCCACCCAGGCCAAGCGCCGCGTGTTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGC
GCCCTGTTTCATCGGCTTCCTGGGCGCCGCCGGGAGCACCATGGGCGCCGCCCTCCGTGACCCCTG
ACCGTGCAAGGCCCGCCAGCTGCTGAGCGGCATCGTGACGAGCAGACAACACCTGCTGCGCGC
CATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTTGGGGCATCAAGCAGCTGCAGGCC
GCATCCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGC
GGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGAC
CGAGATCTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCC
TGATCTACAACCTGATCGAGATCGCCAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTG
GAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGGCTGTGGTACATC
CGCATCTTCATCATGATCGTGCGGCCCTGATCGGCCTGCGCATCGTGTTCCCGTGCTGAGC
ATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGCTGCCCGCCAG
CGCGGCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGGACCGCGACCGCA
GCAACCGCCTGGTGACGGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCTGT
TCAGCTACCAACCGCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAGCTGCTGGGCC
GCCGCGGCTGGGAGGCCCTGAAGTACTGGTGGAACTGCTGCAGTACTGGAGCCAGGAGCTG
AAGAGCAGCGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCG
CATCATCGAGATCGTGACGCGCATCTTCCGCGCCGTGATCCACATCCCCCGCCGATCCGCCA
GGGCCTGGAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA

FIG. 52 (SEQ ID NO:65)

gp160.mod.US4.delV2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGC
TGCCCGTGTTGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTAC
AAGGCCGAGGCCCAACAGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCC
CCAGGAGGTGAACCTGACCAACGTGACCGAGAATTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGACCTGAACTGCACCGACAAGCTGACCGGCAGCACCA
CGGCACCAACAGCACCAGCGGCACCAACAGCACCAGCGGCACCAACAGCACCAGCACC
ACAGCACCGACAGCTGGGAGAAGATGCCCCAGGGCGAGATCAAGAACTGCAGCTTCAAC
ATCGGCGCCGGCCGCTGATCAACTGCAACACCAGCGTGATCACCAGGCCTGCCCCAA
GGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTCGCCATCCTGA
AGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGACG
TGCACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGC
CGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCAACGACAACGCCAAGACCATCATCG
TGCAGCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGTAAG
AGCATCCACATCGGCCCCCGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCGACAT
CCGCCAGGCCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAGATCG
TGGAGAAGCTGCGCGAGCAGTTCCGCAACAACAAGACCATCATCTTCAACAGCAGCAGC
GGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTG
CAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGAACAAGACCA
AGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG
GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAA
TATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCAACGACA
CCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGTAC
AAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGCGCCCCACCCAGGCCAAGCGCCG
CGTGGTGACGCGGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGTTTCATCGGCTTCTTG
GCGCCGCGGGAGACCATGGGCGCCGCTCCGTGACCTGACCGTGACGGCCCGCCAG
CTGCTGAGCGGCATCGTGACGAGCAGAACAACTGCTGCGCGCCATCGAGGCCAGCA
GCACCTGCTGACGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCG
TGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTG
ATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGAT
CTGGGACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGA
TCTACAACCTGATCGAGATCGCCAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTG
GAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGGCTGTGGTA
CATCCGCATCTTCATCATGATCGTGGGCGGCCTGATCGGCCTGCGCATCGTGTTCCCG
TGCTGAGCATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGC
CTGCCCCGCCAGCGCGGCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCG
CGACCGCGACCGCAGCAACCGCCTGGTGACGGCCTGCTGGCCCTGATCTGGGACGACC
TGCGCAGCCTGTGCCTGTTTCAGCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGGCC
CGCATCGTGAGCTGCTGGGCGCGCGGCTGGGAGGCCCTGAAGTACTGGTGAACCT
GCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTCAACGCCACCG
CCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGATCGTGACGCGCATCTTCCGC
GCCGTGATCCACATCCCCCGCCGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGA
TATCGGATCCTCTAGA

FIG. 53 (SEQ ID NO:66)

gp160.modUS4delV1/2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAATAACGGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGCGCGCCGCGCCAGGCCTGCC
CAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCGGCTTCGCCATCCTGAA
GTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGCAAGTGCA
CCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAG
GAGATCGTGCTGCGCTCCGAGAACTTCAACGACAACGCCAAGACCATCATCGTGACAGCTGAA
CGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCG
GCCCCGCGCGCCTTCTACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCA
ACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAGATCGTGGAAGAAGCTGCGCGAGCAG
TTCGGAACAACAAGACCATCATCTTCAACAGCAGCAGCGGCGGCGACCCCGAGATCGTGTT
CCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACACCAGCCAGCTGTTCAACAGCAC
CTGGAACATCACCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCC
GCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATC
CGCGGCCAGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGACCCGCGACGGCGGCAC
CAACAACAACCGCACCAACGACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACA
ACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGGCCCCC
ACCCAGGCCAAGCGCCGCGTGGTGAGCGCGGAGAAGCGCGCCGTGGGCGCTGGGCGCCCTGTT
CATCGGCTTCTGGGCGCCGCCGGGAGCACCATGGGCGCCGCTCCGTGACCCCTGACCGTGCA
GGCCCGCCAGCTGCTGAGCGGCATCGTGAGCAGCAGAGAACAACCTGCTGCGCGCCATCGAGG
CCCAGCAGCACCTGCTGACGCTGACCGTGTTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTG
GCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCT
GATCTGCACCACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCT
GGGACAACATGACCTGGATGGAGTGGGAGCGGAGATCGGCAACTACACCGGCCTGATCTAC
AACCTGATCGAGATCGCCCAGAACAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGG
ACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCAACTGGCTGTGGTACATCCGCATCT
TCATCATGATCGTGGGCGGCCCTGATCGGCCTGCGCATCGTGTTCCGCCGTGCTGAGCATCGTGA
ACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGCTGCCCGCCAGCGCGGC
CCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCAGCAACC
GCCTGGTGACGGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCTGTTCAGCT
ACCACCGCCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGAGCTGCTGGGCCCGCCGCG
GCTGGGAGGCCCTGAAGTACTGGTGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGC
AGCGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCATC
GAGATCGTGACGCGCATCTCCGCGCCGTGATCCACATCCCCCGCCGCATCCGCCAGGGCCTG
GAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA

FIG. 54 (SEQ ID NO:67)

gp160.modUS4 del 128-194

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCA
GTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGCGTGCCCGTG
TGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAGGCCGAGGC
CCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAGGAGGTGAACC
TGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAGCAGATGCATGAG
GACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAAGCTGACCCCCCTGTGCGTG
GGGGCAGGGAAGTGCAGAGACCAGCGTGATCACCCAGGCCTGCCCAAGGTGAGCTTCGAGCC
CATCCCCATCCACTACTGCGCCCCCGCGGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTT
CAACGGCACCGGCCCTGCAAGAACGTGAGCACCGTGCAAGTGACCCACGGCATCCGCCCCG
TGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCC
GAGAACTTCACCGACAACGCCAAGACCATCATCGTGACAGTGAACGAGTCCGTGGAGATCAA
CTGCATCCGCCCCAACAACAACACGCGTAAGAGCATCCACATCGGCCCGGCGCGCCTTCTA
CGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCACTGCAACATCAGCAAGGCCAACT
GGACCAACACCCCTCGAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACC
ATCATCTTCAACAGCAGCAGCGCGGCCGACCCGAGATCGTGTTCCACAGCTTCAACTGCGGC
GGCGAGTTCCTTCTACTGCAACACCGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAG
GTGAACAAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAA
CATGTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCATCCGCGGCCAGATCAAGTGCA
GCAGCAATATTACCGGCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCAAC
GACACCGAGACCTTCGCCCCGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGTA
CAAGTACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGCCCCCAACCGGCCAAGCGCCGCG
TGGTGACGCGGAGAAGCGCGCCGTGGGCTGGGCGCCCTGTTTCATCGGCTTCCTGGGCGCCG
CCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACGGCCCGCCAGCTGCTGAGC
GGCATCGTGACGAGCAGAACAACCTGCTGCGCGCCATCGAGGCCCGAGCAGCACCTGCTGCA
GCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGCTACCTGA
AGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGCAAGCTGATCTGCACCACCACCGTG
CCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAACATGACCTGGAT
GGAGTGGGAGCGGAGATCGGCAACTACACCGGCTGATCTACAACCTGATCGAGATCGCCC
AGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTG
GAACTGGTTCGACATACCAACTGGCTGTGGTACATCCGCATCTTCATCATGATCGTGGGCGG
CCTGATCGGCTGCGCATCGTGTTCCCGTGCTGAGCATCGTGAACCGCGTGCGCCAGGGCTA
CAGCCCCATCAGCCTGCAGACCCGCTGCCCGCCAGCGCGGCCCGACCGCCCCGAGGGCA
TCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCAGCAACCGCCTGGTGCACGGCCTGCTG
GCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCTGTTACAGTACCACCGCCTGCGCGACCTG
CTGCTGATCGTGGCCCGCATCGTGGAGCTGCTGGGCCGCGCGGCTGGGAGGCCCTGAAGTAC
TGGTGGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTCAA
CGCCACCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGATCGTGACGCGCATCTT
CCGCGCCGTGATCCACATCCCCCGCCGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGA
TATCGGATCCTCTAGA

FIG. 55 (SEQ ID NO:68)

Env_US4_C4wt

GACACTATCATACTCCCATGCAGAATAAGACAAATTATAAACATGTGGCAAGAAGTAGG
AAAAGCAATGTATGCCCCCTCCCATCAGAGGACAAATTAAATGTTTCATCAAATATTACAG
GGCTGCTATTAAGTAGAGATGGTGGT

FIG. 56 (SEQ ID NO:69)

650621 " 675460

Env_SF162_C4wt

GGAACTATCACA CTCCCATGCAGAATAAAACAAATTATAAACAGGTGGCAGGAAGTAGG
AAAAGCAATGTATGCCCCCTCCCATCAGAGGACAAATTAGATGCTCATCAAATATTACAG
GACTGCTATTAACAAGAGATGGTGGT

FIG. 57 (SEQ ID NO:70)

650527 " 2423460

Env_US4_C4mod

GACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAGGAGGTGGG
CAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAACATCACCG
GCCTGCTGCTGACCCGCGACGGCGGC

FIG. 58 (SEQ ID NO:71)

Env_SF162_C4mod

GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCAGGAGGTGGG
CAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCGCTGCAGCAGCAACATCACCG
GCCTGCTGCTGACCCGCGACGGCGGC

FIG. 59 (SEQ ID NO:72)

660661 5153460

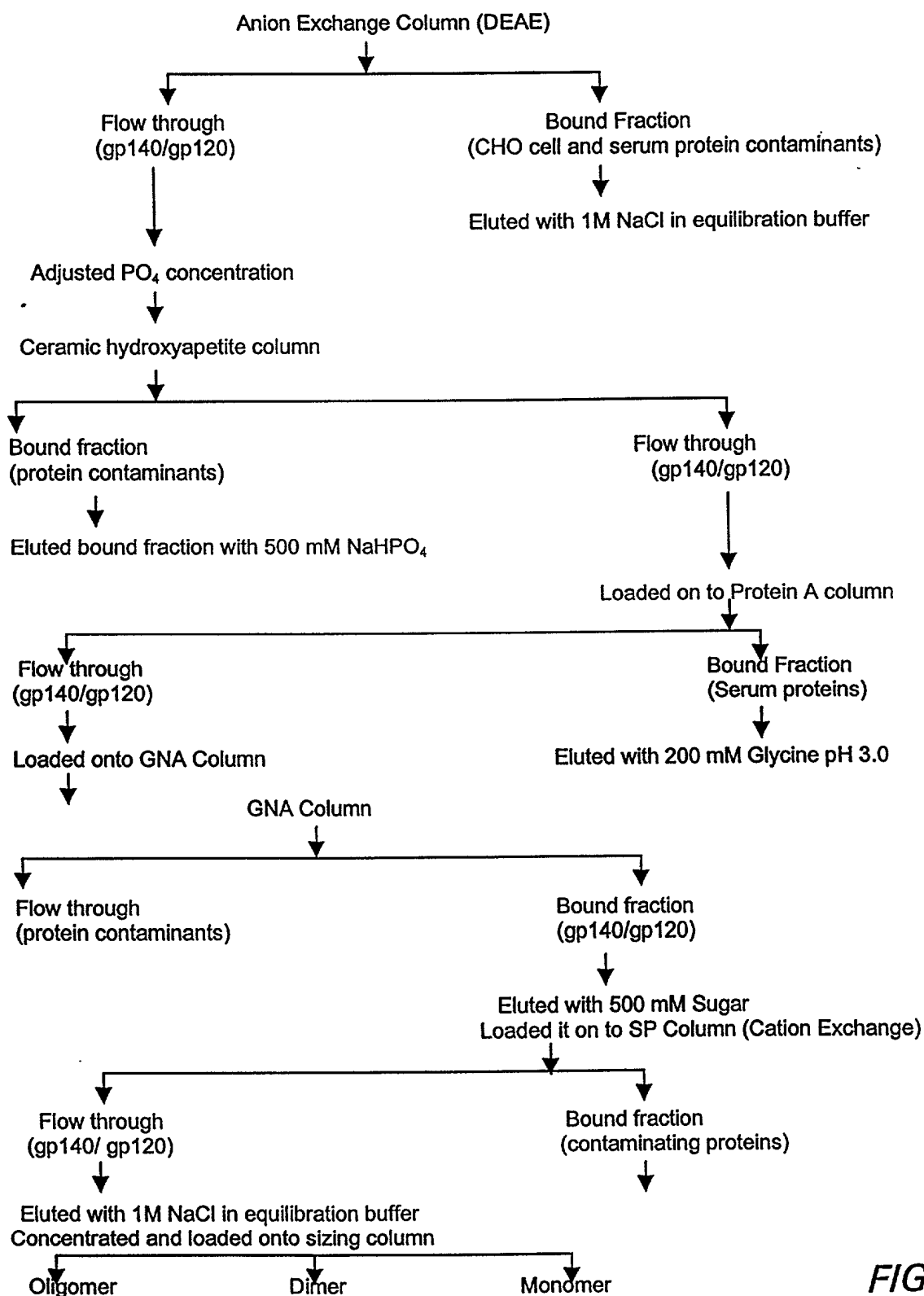


FIG. 60.

gp160mod.us4.gag.modSF2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGA
 GCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTG
 CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAG
 GCGGAGGCCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG
 GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG
 CAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG
 ACCCCCCCTGTGCGTGACCCTGAACTGCACCGACAAGCTGACCGGCAGCACCAACGGCACC
 AACAGCACCAGCGGCACCAACAGCACCAGCGGCACCAACAGCACCAGCACCAACAGCACC
 GACAGCTGGGAGAAGATGCCCCAGGGCGAGATCAAGAACTGCAGCTTCAACATCAACACC
 AGCGTGCGCGACAAGGTGCAGAAGGAGTACAGCCTGTTCTACAAGCTGGACGTGGTGCCC
 ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCAACACCAGCGTGATCACCCAG
 GCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCCGGCTTC
 GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAACGTGAGC
 ACCGTGCAGTGACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC
 AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTCACCGACAACGCCAAGACC
 ATCATCGTGAGCTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAACAACACG
 CGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGC
 GACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG
 ATCGTGAGGAAGCTGCGCGAGCAGTTCCGCAACAACAAGACCATCATCTTCAACAGCAGC
 AGCGGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC
 TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGAACAAGACC
 AAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG
 GAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGCAGCAAT
 ATTACCGGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC
 GAGACCTTCCGCCCCGGCGGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTGTACAAG
 TACAAGGTGGTGCGCATCGAGCCCCCTGGGCGTGGCCCCACCCAGGCCAAGCGCCGCGTG
 GTGCAGCGCGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGTTTCATCGGCTTCTTGGGCGCC
 GCCGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCGTGACGGCCCCGCCAGCTGCTG
 AGCGGCATCGTGACGAGCAGAAACAACCTGCTGCGCGCCATCGAGGCCAGCAGCACCTG
 CTGCAGCTGACCGTGTGGGCATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTGGAGCGC
 TACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGCAAGCTGATCTGCACC
 ACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAAC
 ATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTACAACCTG
 ATCGAGATCGCCCAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTGGACAAG
 TGGGCCAGCCTGTGGAACCTGGTTCGACATCACCACCTGGCTGTGGTACATCCGCATCTTC
 ATCATGATCGTGGGCGGCCTGATCGGCCTGCGCATCGTGTTTCGCCGTGCTGAGCATCGTG
 AACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGCCTGCCCGCCCAGCGC
 GGCCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACCGCAGC
 AACCGCCTGGTGCACGGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTGTGCCTG
 TTCAGCTACCACCGCCTGCGCGACCTGCTGCTGATCGTGCCCGCATCGTGAGAGCTGCTG
 GGCCGCCGCGGCTGGGAGGCCCTGAAGTACTGGTGAACCTGCTGCAGTACTGGAGCCAG
 GAGCTGAAGAGCAGCGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCCGAGGGC
 ACCGACCGCATCATCGAGATCGTGACGCGCATCTTCCGCGCCGTGATCCACATCCCCCGC
 CGCATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGAGAATTC

FIG. 61 (SEQ ID NO:73)

CGCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCCTAACGTTACTGGCCGAAGCCGC
 TTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTT
 GGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCTAGGGGTCTT
 TCCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTG
 GAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCCTTTCAGGCAGCGGAACCCCCCA
 CCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATAACCTGCAAAGGCG
 GCACAACCCCAAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCC
 TCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTTGTATGGGATCT
 GATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTA
 GGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATAATACCATGGGCGC
 CCGCGCCAGCGTGCTGAGCGCGCGGAGCTGGACAAGTGGGAGAAGATCCGCCTGCGCCC
 CGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGGGCCAGCCGCGAGCTGGAGCG
 CTTGCGCGTGAACCCCGGCTGCTGGAGACCAGCGAGGGCTGCCGCCAGATCCTGGGCCA
 GCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGCAGCCTGTACAACACCGTGCG
 CACCCTGTACTGCGTGACACGCGCATCGACGTCAAGGACACCAAGGAGGCCCTGGAGAA
 GATCGAGGAGGAGCAGAACAAGTCCAAGAAGAAGGCCAGCAGGCCGCGCCGCGCCGCGG
 CACCGGCAACAGCAGCCAGGTGAGCCAGAACTACCCCATCGTGCAGAACCTGCAGGGCCA
 GATGGTGCACCAGGCCATCAGCCCCCGCACCCTGAACGCCTGGGTGAAGGTGGTGGAGGA
 GAAGGCCTTCAGCCCCGAGGTGATCCCATGTTTCAGCGCCCTGAGCGAGGGCGCCACCCC
 CCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCAGATGCT
 GAAGGAGACCATCAACGAGGAGGCCCGCGAGTGGGACCGCGTGACCCCGTGACGCGCGG
 CCCCATCGCCCCCGGCCAGATGCGCGAGCCCCGCGGCAGCGACATCGCCGGCACCACCAG
 CACCCTGCAGGAGCAGATCGGCTGGATGACCAACAACCCCCCATCCCCGTGGGCGAGAT
 CTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCGTGCGGATGTACAGCCCCACCAG
 CATCCTGGACATCCGCCAGGGCCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGCTTCTA
 CAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGAAGAACTGGATGACCGAGACCCT
 GCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTGAAGGCTCTCGGCCCCGCGGC
 CACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGGCCCCGGCCACAAGGCCCG
 CGTGCTGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCGACCATCATGATGCAGCGCGG
 CAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAACTGCGGCAAGGAGGGCCACAC
 CGCCAGGAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCCGCGAGGGCCA
 CCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCCTGGGCAAGATCTGGCCCAGCTA
 CAAGGGCCGCCCCGGCAACTTCCTGCAGAGCCGCCCCGAGCCCACCGCCCCCCCCGAGGA
 GAGCTTCCGCTTCGGCGAGGAGAAGACCACCCCCAGCCAGAAGCAGGAGCCCATCGACAA
 GGAGCTGTACCCCTGACCAGCCTGCGCAGCCTGTTTCGGCAACGACCCACGAGCCAGTA
 AGAATTCAGACTCGAGCAAGTCTAGA

FIG. 61 (CONT'D.) (SEQ ID NO:73)

gp160mod.SF162.gag.modSF2

GAATTCCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGG
AGCAGTCTTCGTTTCGCCCAGCGCCGTGGAGAAGCTGTGGGTGACCGTGTACTACGGCG
TGCCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCCTAC
GACACCGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCC
CCAGGAGATCGTGCTGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGG
TGGAGCAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTG
AAGCTGACCCCCCTGTGCGTGACCTGCACTGCACCAACCTGAAGAACGCCACCAACAC
CAAGAGCAGCAACTGGAAGGAGATGGACCGCGGCGAGATCAAGAACTGCAGCTTCAAGG
TGACCACCAGCATCCGCAACAAGATGCAGAAGGAGTACGCCCTGTTCTACAAGCTGGAC
GTGGTGCCCATCGACAACGACAACACCAGCTACAAGCTGATCAACTGCAACACCAGCGT
GATCACCACAGGCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCC
CCGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCGGCCCTGC
ACCAACGTGAGCACCGTGCAGTGACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCT
GCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGTGTTGATCCGCAGCGAGAACTTACCG
ACAACGCCAAGACCATCATCGTGAGCTGAAGGAGAGCGTGAGATCAACTGCACCCGC
CCCAACAACAACACCCGCAAGAGCATCACCATCGGCCCCGGCCGCGCCTTCTACGCCAC
CGGCGACATCATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCGGCGAGAAGTGGA
ACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCAGTTTCGGCAACAAGACCATC
GTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGTGATGCACAGCTTCAACTGCGG
CGGCGAGTTCTTCTACTGCAACAGCACCCAGCTGTTCAACAGCACCTGGAACAACACCA
TCGGCCCCAACAACACCAACGGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATC
AACCGCTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCG
CTGCAGCAGCAACATCACCGGCTGCTGCTGACCCGCGACGGCGGCAAGGAGATCAGCA
ACACCACCGAGATCTTCCGCCCCGGCGGCGGCGACATGCGCGACAACCTGGCGCAGCGAG
CTGTACAAGTACAAGGTGGTGAAGATCGAGCCCCCTGGGCGTGGCCCCACCAAGGCCAA
GCGCCGCGTGGTGACGCGGAGAAGCGCGCCGTGACCCTGGGCGCCATGTTCTTGGGCT
TCCTGGGCGCCCGCGGCGAGCACCATGGGCGCCCGCAGCCTGACCCTGACCGTGAGGCC
CGCCAGCTGCTGAGCGGCATCGTGAGCAGCAGAACAACTGCTGCGCGCCATCGAGGC
CCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAGGCCCGCGTG
TGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGCAGCGGC
AAGCTGATCTGCACCACCGCCGTGCCCTGGAACGCCAGCTGGAGCAACAAGAGCCTGGA
CCAGATCTGGAACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGACAACCTACACCA
ACCTGATCTACACCCTGATCGAGGAGAGCCAGAACCAGCAGGAGAAGAACGAGCAGGAG
CTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAAGTGGCT
GTGGTACATCAAGATCTTCATCATGATCGTGGGCGGCCTGGTGGGCCTGCGCATCGTGT
TCACCGTGCTGAGCATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCCTGAGCTTCCAG
ACCCGCTTCCCCGCCCCCGCGGCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGG
CGAGCGCGACCGCGACCGCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGGG
ACGACCTGCGCAGCCTGTGCCTGTTTACGCTACCACCGCCTGCGCGACCTGATCCTGATC
GCCGCCCGCATCGTGGAGCTGCTGGGCCGCGCGGCTGGGAGGCCCTGAAGTACTGGGG
CAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGCGCCGTGAGCCTGTTTCGACG
CCATCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGGTGGCCAGCGCATC
GGCCGCGCCTTCTGTCACATCCCCCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCT

FIG. 62 (SEQ ID NO:74)

GTAAC TCGAGCAAGTCTAGAGAATTCCGCCCCCCCCCCCCCCCCCTCTCCCTCCCCC
 CCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATAT
 GTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGAAACCTGGCCCTG
 TCTTCTTGACGAGCATTCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTG
 TTGAATGTCGTGAAGGAAGCAGTTCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGT
 AGCGACCCTTTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAA
 AGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGT
 TGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAA
 GGATGCCCAGAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCT
 TTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTG
 GTTTTCCTTTGAAAAACACGATAATACCATGGGCGCCCGCGCCAGCGTGCTGAGCGGCG
 GCGAGCTGGACAAGTGGGAGAAGATCCGCCTGCGCCCCGGCGGCAAGAAGAAGTACAAG
 CTGAAGCACATCGTGTGGGCCAGCCGCGAGCTGGAGCGCTTCGCCGTGAACCCCGGCCT
 GCTGGAGACCAGCGAGGGCTGCCGCCAGATCCTGGGCCAGCTGCAGCCCAGCCTGCAGA
 CCGGCAGCGAGGAGCTGCGCAGCCTGTACAACACCGTGGCCACCCTGTACTGCGTGCAC
 CAGCGCATCGACGTCAAGGACACCAAGGAGGCCCTGGAGAAGATCGAGGAGGAGCAGAA
 CAAGTCCAAGAAGAAGGCCCAGCAGGCCGCGCCGCGCCGCGCCGCGCACCGGCAACAGCAGCC
 AGGTGAGCCAGAACTACCCCATCGTGCAGAACCTGCAGGGCCAGATGGTGCACCAGGCC
 ATCAGCCCCCGCACCCCTGAACGCCTGGGTGAAGGTGGTGGAGGAGAAGGCCTTCAGCCC
 CGAGGTGATCCCCATGTTTACGCGCCCTGAGCGAGGGCGCCACCCCCAGGACCTGAACA
 CGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCAGATGCTGAAGGAGACCATC
 AACGAGGAGGCCCGCCGAGTGGGACCGCGTGACCCCGTGACGCGCGGCCCATCGCCCC
 CGGCCAGATGCGCGAGCCCCGCGGCAGCGACATCGCCGGCACCACCAGCACCCCTGCAGG
 AGCAGATCGGCTGGATGACCAACAACCCCCCATCCCCGTGGGCGAGATCTACAAGCGG
 TGGATCATCCTGGGCCTGAACAAGATCGTGCGGATGTACAGCCCCACCAGCATCCTGGA
 CATCCGCCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCGCTTCTACAAGACCC
 TGCGCGCTGAGCAGGCCAGCCAGGACGTGAAGAACTGGATGACCGAGACCCTGCTGGTG
 CAGAACGCCAACCCCGACTGCAAGACCATCCTGAAGGCTCTCGGCCCCGCGGCCACCCT
 GGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGGCCCGGCCACAAGGCCCGCGTGC
 TGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCGACCATCATGATGCAGCGCGGCAAC
 TTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAACTGCGGCAAGGAGGGCCACACCGC
 CAGGAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCCGCGAGGGCCACC
 AGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCTGGGCAAGATCTGGCCCAGCTAC
 AAGGGCCGCCCCGGCAACTTCTTGCAGAGCCGCCCCGAGCCACCGCCCCCCCCGAGGA
 GAGCTTCCGCTTCGGCGAGGAGAAGACCACCCCGAGCCAGAAGCAGGAGCCCATCGACA
 AGGAGCTGTACCCCTGACCAGCCTGCGCAGCCTGTTCCGGCAACGACCCCGAGCAGCCAG
 TAAGAATTCAGACTCGAGCAAGTCTAGA

FIG. 62 (CONT'D.) (SEQ ID NO:74)

gp160modUS4.delV1/V2.gag.modSF2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGA
GCAGTCTTCGTTTCGCCCAGCGCCACCACCGTGCTGTGGGTGACCGTGTAACGGCGTG
CCCGTGTTGAAGGAGGCCACCACCACCTGTTCTGCGCCAGCGACGCCAAGGCTTACAAG
GCCGAGGCCCAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCGAG
GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG
CAGATGCATGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGGGCGCC
GGCCAGGCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCCCGCC
GGCTTCGCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCGGCCCTGCAAGAAC
GTGAGCACCGTGCAAGTGACCCACGGCATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTG
AACGGCAGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACTTACCCGACAACGCC
AAGACCATCATCGTGCAAGTGAACGAGTCCGTGGAGATCAACTGCATCCGCCCCAACAAC
AACACGCGTAAGAGCATCCACATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGACATC
ATCGGCGACATCCGCCAGGCCCACTGCAACATCAGCAAGGCCAACTGGACCAACACCCTC
GAGCAGATCGTGGAGAAGCTGCGCGAGCAGTTCGGCAACAACAAGACCATCATCTTCAAC
AGCAGCAGCGCGCGGCGACCCCGAGATCGTGTTCCACAGCTTCAACTGCGGCGGCGAGTTC
TTCTACTGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCACCGAGGAGGTGAAC
AAGACCAAGGAGAACGACACCATCATCCTGCCCTGCCGCATCCGCCAGATCATCAACATG
TGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCAAGTGCAGC
AGCAATATTACCGCCTGCTGCTGACCCGCGACGGCGGCACCAACAACAACCGCACCAAC
GACACCGAGACCTTCCGCCCCGGCGGCGGCAACATGAAGGACAACCTGGCGCAGCGAGCTG
TACAAGTACAAGGTGGTGCGCATCGAGCCCCTGGGCGTGCGCCCCACCCAGGCCAAGCGC
CGCGTGCTGAGCGCGGAGAAGCGCGCCGTGGGCCTGGGCGCCCTGTTTCATCGGCTTCCTG
GGCGCCGCGGGGAGCACCATGGGCGCCGCTCCGTGACCCTGACCCTGACCGTGCAGGCCCGCCAG
CTGCTGAGCGGCATCGTGCAAGCAGCAGAACAACCTGCTGCGCGCCATCGAGGCCAGCAG
CACCTGCTGCAAGCTGACCGTGTTGGGCGATCAAGCAGCTGCAGGCCCGCATCCTGGCCGTG
GAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCGATCTGGGCGTGCAGCGGCAAGCTGATC
TGCAACACCACCGTGCCCTGGAACAGCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGG
GACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTACACCGGCCTGATCTAC
AACCTGATCGAGATCGCCAGAACCAGCAGGAGAAGAACGAGCAGGAGCTGCTGGAGCTG
GACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAACCAACTGGCTGTGGTACATCCGC
ATCTTCATCATGATCGTGGGCGGCTGATCGGCCTGCGCATCGTGTTCCGCGTGCTGAGC
ATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCATCAGCCTGCAGACCCGCTGCCCGCC
CAGCGCGGCCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGGACCGCGAC
CGCAGCAACCGCCTGGTGACGGCCTGCTGGCCCTGATCTGGGACGACCTGCGCAGCCTG
TGCCCTGTTTACGCTACACCGCCTGCGCGACCTGCTGCTGATCGTGGCCCCGATCGTGGAG
CTGCTGGGCCCGCGCGGCTGGGAGGCCCTGAAGTACTGGTGGAACCTGCTGCAGTACTGG
AGCCAGGAGCTGAAGAGCAGCGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC
GAGGGCACCGACCGCATCATCGAGATCGTGACGCGCATCTTCCGCGCCGTGATCCACATC
CCCCGCCGATCCGCCAGGGCCTGGAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA
GAATTCGCCCCCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCCTAACGTTACTGGCCGA
AGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCG
TCTTTTGGCAATGTGAGGGCCCGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGG
GGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTT

FIG. 63 (SEQ ID NO:75)

CCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCCTTTGCAGGCAGCGGAAC
 CCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCA
 AAGGCGGCACAACCCCAAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGG
 CTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTTGTATG
 GGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAA
 CGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTGAAAAACACGATAATACCAT
 GGGCGCCCCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGTGGGAGAAGATCCGCCT
 GCGCCCCGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGGGCCAGCCGCGAGCT
 GGAGCGCTTCGCCGTGAACCCCGGCCTGCTGGAGACCAGCGAGGGCTGCCGCCAGATCCT
 GGGCCAGCTGCAGCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGCAGCCTGTACAACAC
 CGTGGCCACCCTGTACTGCGTGCACCAGCGCATCGACGTCAAGGACACCAAGGAGGCCCT
 GGAGAAGATCGAGGAGGAGCAGAACAAGTCCAAGAAGAAGGCCCAGCAGGCCGCCGCCGC
 CGCCGGCACCCGGCAACAGCAGCCAGGTGAGCCAGAACTACCCCATCGTGCAGAACCTGCA
 GGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCCCTGAACGCCTGGGTGAAGGTGGT
 GGAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCCCTGAGCGAGGGCGC
 CACCCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCACCAGGCCGCCATGCA
 GATGCTGAAGGAGACCATCAACGAGGAGGCCGCCGAGTGGGACCGCGTGCACCCCGTGCA
 CGCCGGCCCCCATCGCCCCCGGCCAGATGCGCGAGCCCCGCGGCAGCGACATCGCCGGCAC
 CACCAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCAACAACCCCCCATCCCCGTGGG
 CGAGATCTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCGTGCAGGATGTACAGCCC
 CACCAGCATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCCGCGACTACGTGGACCG
 CTTCTACAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGAAGAACTGGATGACCGA
 GACCCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTGAAGGCTCTCGGCCC
 CGCGGCCACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGGCCCCGGCCACAA
 GGCCCGCGTGCTGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCGACCATCATGATGCA
 GCGCGGCAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAACTGCGGCAAGGAGGG
 CCACACCGCCAGGAACTGCCGCGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCCGCGA
 GGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCCTGGGCAAGATCTGGCC
 CAGCTACAAGGGCCGCCCGGCAACTTCCTGCAGAGCCGCCCGAGCCCACCGCCCCCCC
 CGAGGAGAGCTTCCGCTTCGGCGAGGAGAAGACCACCCCCAGCCAGAAGCAGGAGCCCAT
 CGACAAGGAGCTGTACCCCTGACCAGCCTGCGCAGCCTGTTTCGGCAACGACCCAGCAG
 CCAGTAAGAATTACAGACTCGAGCAAGTCTAGA

FIG. 63 (CONT'D.) (SEQ ID NO:75)

gp160.modSF162.delV2.gag.modSF2

GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGA
GCAGTCTTCGTTTTCGCCAGCGCCGTGGAGAAGCTGTGGGTGACCGTGTACTACGGCGTG
CCCGTGTGGAAGGAGGCCACCACCACCCTGTTCTGCGCCAGCGACGCCAAGGCCTACGAC
ACCGAGGTGCACAACGTGTGGGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCCAG
GAGATCGTGCTGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG
CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG
ACCCCCCTGTGCGTGACCCCTGCACTGCACCAACCTGAAGAACGCCACCAACACCAAGAGC
AGCAACTGGAAGGAGATGGACCGCGGCGAGATCAAGAACTGCAGCTTCAAGGTGGGCGCC
GGCAAGCTGATCAACTGCAACACCAGCGTGATCACCCAGGCCTGCCCCAAGGTGAGCTTC
GAGCCCATCCCCATCCACTACTGCGCCCCCGCGGCTTCGCCATCCTGAAGTGCAACGAC
AAGAAGTTCAACGGCAGCGGCCCTGCACCAACGTGAGCACCGTGCAGTGCACCCACGGC
ATCCGCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGTG
GTGATCCGCAGCGAGAACTTCAACGACAACGCCAAGACCATCATCGTGACGCTGAAGGAG
AGCGTGAGATCAACTGCACCCGCCCCAACAACAACACCCGCAAGAGCATCACCATCGGC
CCCGGCCGCGCCTTCTACGCCACCGGCGACATCATCGGCGACATCCGCCAGGCCCACTGC
AACATCAGCGGCGAGAAAGTGGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCC
CAGTTCGGCAACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGTG
ATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCCAGCTGTTCAAC
AGCACCTGGAACAACACCATCGGCCCAACAACACCAACGGCACCATCACCTGCCCTGC
CGCATCAAGCAGATCATCAACCGCTGGCAGGAGGTGGGCAAGGCCATGTACGCCCCCCCC
ATCCGCGGCCAGATCCGCTGCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGC
GGCAAGGAGATCAGCAACACCACCGAGATCTTCCGCCCCGGCGGCGGCGACATGCGCGAC
AACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCCCTGGGCGTGCC
CCCACCAAGGCCAAGCGCCGCGTGTTGTCAGCGCGAGAAGCGCGCCGTGACCCTGGGCGCC
ATGTTCTTGGGCTTCTGGGCGCCGCGGCGAGCACCATGGGCGCCCGCAGCCTGACCCTG
ACCGTGCAGGCCCGCCAGCTGCTGAGCGGCATCGTGACGAGCAGAGAACAACCTGCTGCGC
GCCATCGAGGCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCAG
GCCCCGCTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGC
TGCAGCGGCAAGCTGATCTGCACCACCGCCGTGCCCTGGAACGCCAGCTGGAGCAACAAG
AGCCTGGACCAGATCTGGAACAACATGACCTGGATGGAGTGGGAGCGCGAGATCGACAAC
TACACCAACCTGATCTACACCCTGATCGAGGAGAGCCAGAACCAGCAGGAGAAGAACGAG
CAGGAGCTGCTGGAGCTGGACAAGTGGGCCAGCCTGTGGAACCTGGTTCGACATCAGCAAG
TGGCTGTGGTACATCAAGATCTTCATCATGATCGTGGGCGGCCTGGTGGGCCTGCGCATC
GTGTTACCGTGCTGAGCATCGTGAACCGCGTGCGCCAGGGCTACAGCCCCCTGAGCTTC
CAGACCCGCTTCCCCGCCCCCGCGGCCCGACCGCCCCGAGGGCATCGAGGAGGAGGGC
GGCGAGCGCGACCGCGACCGCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGG
GACGACCTGCGCAGCCTGTGCCTGTTTACGCTACCACCGCCTGCGCGACCTGATCCTGATC
GCCGCCCGCATCGTGAGCTGCTGGGCCGCCGCGGCTGGGAGGCCCTGAAGTACTGGGGC
AACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGCGCCGTGAGCCTGTTTCGACGCC
ATCGCCATCGCCGTGGCCGAGGGCACCGACCGCATCATCGAGGTGGCCCAGCGCATCGGC
CGCGCCTTCTGACATCCCCCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCTGTAA
CTCGAGCAAGTCTAGAGAATTCCGCCCCCCCCCCCCCCCCCTCTCCCTCCCCCCCCC
TAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATT
TTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTT

FIG. 64 (SEQ ID NO:76)

GACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGT
 CGTGAAGGAAGCAGTTCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCCT
 TTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGT
 ATAAGATACACCTGCAAAGGCGGCACAACCCCAAGTGCCACGTTGTGAGTTGGATAGTTGT
 GGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAA
 GGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTA
 GTCGAGGTTAAAAAACGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTTTTGAAA
 AACACGATAATACCATGGGCGCCCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGT
 GGGAGAAGATCCGCCTGCGCCCCGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGT
 GGGCCAGCCGCGAGCTGGAGCGCTTCGCCGTGAACCCCGGCCTGCTGGAGACCAGCGAGG
 GCTGCCGCCAGATCCTGGGCCAGCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGC
 GCAGCCTGTACAACACCGTGGCCACCCTGTACTGCGTGCACCAGCGCATCGACGTCAAGG
 ACACCAAGGAGGCCCTGGAGAAGATCGAGGAGGAGCAGAACAAGTCCAAGAAGAAGGCC
 AGCAGGCCCGCCGCCCGCCCGGCACCGGCAACAGCAGCCAGGTGAGCCAGAACTACCCCA
 TCGTGCAGAACCTGCAGGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCTGAACG
 CCTGGGTGAAGGTGGTGGAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCG
 CCCTGAGCGAGGGCGCCACCCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCC
 ACCAGGCCGCCATGCAGATGCTGAAGGAGACCATCAACGAGGAGGCCCGCGAGTGGGACC
 GCGTGCACCCCGTGCACGCCGGCCCCATCGCCCCCGGCCAGATGCGCGAGCCCCGCGGCA
 GCGACATCGCCGGCACCACCAGCACCTGCAGGAGCAGATCGGCTGGATGACCAACAACC
 CCCCCATCCCCGTGGGCGAGATCTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCG
 TGCGGATGTACAGCCCCACCAGCATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCC
 GCGACTACGTGGACCGCTTCTACAAGACCTGCGCGCTGAGCAGGCCAGCCAGGACGTGA
 AGAACTGGATGACCGAGACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCC
 TGAAGGCTCTCGGCCCCGCGGCCACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGG
 GCGGCCCGGCCACAAGGCCCGCGTGCTGGCCGAGGCGATGAGCCAGGTGACGAACCCGG
 CGACCATCATGATGCAGCGCGGCAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCA
 ACTGCGGCAAGGAGGGCCACACCGCCAGGAAGTGC CGCGCCCCCGCAAGAAGGGCTGCT
 GCGCTGCGGCCGCGAGGGCCACCAGATGAAGGACTGCACCGAGCGCCAGGCCAACTTCC
 TGGGCAAGATCTGGCCCAGCTACAAGGGCCGCCCGGCAACTTCTGCAGAGCCGCCCCG
 AGCCACCGCCCCCCCCGAGGAGAGCTTCCGCTTCGGCGAGGAGAAGACCACCCCGAGCC
 AGAAGCAGGAGCCATCGACAAGGAGCTGTACCCCTGACCAGCCTGCGCAGCCTGTTCG
 GCAACGACCCAGCAGCCAGTAAGAATTCAGACTCGAGCAAGTCTAGA

FIG. 64 (CONT'D.) (SEQ ID NO:76)

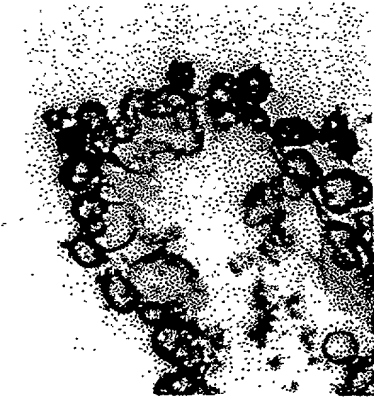


FIG. 65A



FIG. 65B

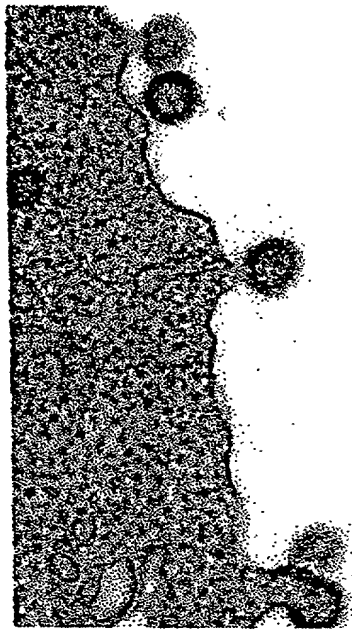


FIG. 65C

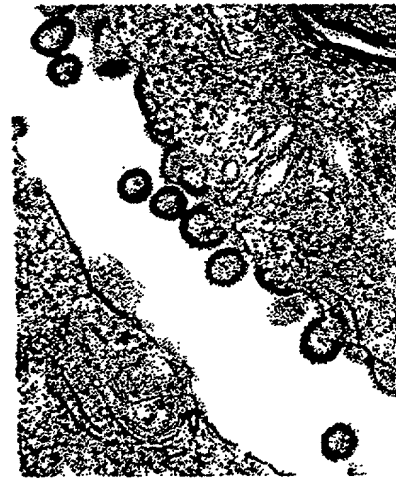


FIG. 65D

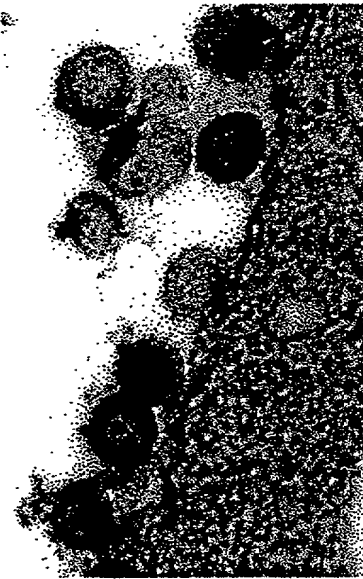


FIG. 65E

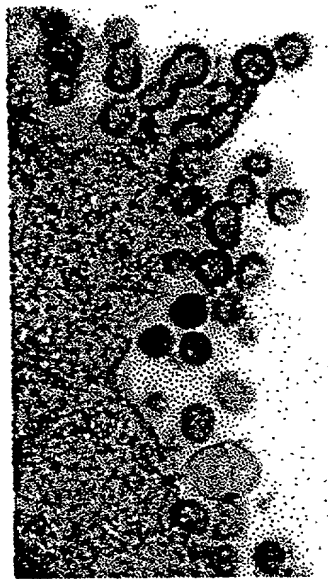


FIG. 65F

		1	100
gp160.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp160.modSF162.delV2	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp160.modSF162.delV1V2	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp140.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp140.mut.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp140.mut7.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp140.mut8.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
gp120.modSF162	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
Consensus	(1)	GAATTCGCCACCATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCT	
		51	100
gp160.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCAGCGCCGTGGAGAAGCTGTGGG	
gp160.modSF162.delV2	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCAGCGCCGTGGAGAAGCTGTGGG	
gp160.modSF162.delV1V2	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCAGCGCCGTGGAGAAGCTGTGGG	
gp140.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCAGCGCCGTGGAGAAGCTGTGGG	
gp140.mut.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCAGCGCCGTGGAGAAGCTGTGGG	
gp140.mut7.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCAGCGCCGTGGAGAAGCTGTGGG	
gp140.mut8.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCAGCGCCGTGGAGAAGCTGTGGG	
gp120.modSF162	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCAGCGCCGTGGAGAAGCTGTGGG	
Consensus	(51)	GCTGTGTGGAGCAGTCTTCGTTTCGCCAGCGCCGTGGAGAAGCTGTGGG	
		101	150
gp160.modSF162	(101)	TGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTG	
gp160.modSF162.delV2	(101)	TGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTG	
gp160.modSF162.delV1V2	(101)	TGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTG	
gp140.modSF162	(101)	TGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTG	
gp140.mut.modSF162	(101)	TGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTG	
gp140.mut7.modSF162	(101)	TGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTG	
gp140.mut8.modSF162	(101)	TGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTG	
gp120.modSF162	(101)	TGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTG	
Consensus	(101)	TGACCGTGTACTACGGCGTGCCCGTGTGGAAGGAGGCCACCACCACCCTG	
		151	200
gp160.modSF162	(151)	TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG	
gp160.modSF162.delV2	(151)	TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG	
gp160.modSF162.delV1V2	(151)	TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG	
gp140.modSF162	(151)	TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG	
gp140.mut.modSF162	(151)	TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG	
gp140.mut7.modSF162	(151)	TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG	
gp140.mut8.modSF162	(151)	TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG	
gp120.modSF162	(151)	TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG	
Consensus	(151)	TTCTGCGCCAGCGACGCCAAGGCCTACGACACCGAGGTGCACAACGTGTG	
		201	250
gp160.modSF162	(201)	GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGATCGTGC	
gp160.modSF162.delV2	(201)	GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGATCGTGC	
gp160.modSF162.delV1V2	(201)	GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGATCGTGC	
gp140.modSF162	(201)	GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGATCGTGC	
gp140.mut.modSF162	(201)	GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGATCGTGC	
gp140.mut7.modSF162	(201)	GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGATCGTGC	
gp140.mut8.modSF162	(201)	GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGATCGTGC	
gp120.modSF162	(201)	GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGATCGTGC	
Consensus	(201)	GGCCACCCACGCCTGCGTGCCACCGACCCCAACCCCAAGGAGATCGTGC	
		251	300
gp160.modSF162	(251)	TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG	
gp160.modSF162.delV2	(251)	TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG	
gp160.modSF162.delV1V2	(251)	TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG	
gp140.modSF162	(251)	TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG	
gp140.mut.modSF162	(251)	TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG	
gp140.mut7.modSF162	(251)	TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG	
gp140.mut8.modSF162	(251)	TGGAGAACGTGACCGAGAACTTCAACATGTGGAAGAACAACATGGTGGAG	

Accession	Gene	Strain	Position	Sequence	Length
gp120.modSF162		(251)	TGGAGAACGTGACCGAGA	301	350
Consensus		(251)	TGGAGAACGTGACCGAGA	301	350
gp160.modSF162		(301)	CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTG		
gp160.modSF162.delV2		(301)	CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTG		
gp160.modSF162.delV1V2		(301)	CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTG		
gp140.modSF162		(301)	CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTG		
gp140.mut.modSF162		(301)	CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTG		
gp140.mut7.modSF162		(301)	CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTG		
gp140.mut8.modSF162		(301)	CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTG		
gp120.modSF162		(301)	CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTG		
Consensus		(301)	CAGATGCACGAGGACATCATCAGCCTGTGGGACCAGAGCCTGAAGCCCTG	351	400
gp160.modSF162		(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCTGCACTGCACCAACCTGAAGA		
gp160.modSF162.delV2		(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCTGCACTGCACCAACCTGAAGA		
gp160.modSF162.delV1V2		(351)	CGTGAAGCTGACCCCCCTGTGCGTG-----		
gp140.modSF162		(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCTGCACTGCACCAACCTGAAGA		
gp140.mut.modSF162		(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCTGCACTGCACCAACCTGAAGA		
gp140.mut7.modSF162		(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCTGCACTGCACCAACCTGAAGA		
gp140.mut8.modSF162		(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCTGCACTGCACCAACCTGAAGA		
gp120.modSF162		(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCTGCACTGCACCAACCTGAAGA		
Consensus		(351)	CGTGAAGCTGACCCCCCTGTGCGTGACCCTGCACTGCACCAACCTGAAGA	401	450
gp160.modSF162		(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGGCGAG		
gp160.modSF162.delV2		(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGGCGAG		
gp160.modSF162.delV1V2		(375)	-----		
gp140.modSF162		(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGGCGAG		
gp140.mut.modSF162		(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGGCGAG		
gp140.mut7.modSF162		(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGGCGAG		
gp140.mut8.modSF162		(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGGCGAG		
gp120.modSF162		(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGGCGAG		
Consensus		(401)	ACGCCACCAACACCAAGAGCAGCAACTGGAAGGAGATGGACCGCGGCGAG	451	500
gp160.modSF162		(451)	ATCAAGAACTGCAGCTTCAAGGTGACCACCAGCATCCGCAACAAGATGCA		
gp160.modSF162.delV2		(451)	ATCAAGAACTGCAGCTTCAAGGTGGGC-----		
gp160.modSF162.delV1V2		(376)	-----GGC-----		
gp140.modSF162		(451)	ATCAAGAACTGCAGCTTCAAGGTGACCACCAGCATCCGCAACAAGATGCA		
gp140.mut.modSF162		(451)	ATCAAGAACTGCAGCTTCAAGGTGACCACCAGCATCCGCAACAAGATGCA		
gp140.mut7.modSF162		(451)	ATCAAGAACTGCAGCTTCAAGGTGACCACCAGCATCCGCAACAAGATGCA		
gp140.mut8.modSF162		(451)	ATCAAGAACTGCAGCTTCAAGGTGACCACCAGCATCCGCAACAAGATGCA		
gp120.modSF162		(451)	ATCAAGAACTGCAGCTTCAAGGTGACCACCAGCATCCGCAACAAGATGCA		
Consensus		(451)	ATCAAGAACTGCAGCTTCAAGGTGACCACCAGCATCCGCAACAAGATGCA	501	550
gp160.modSF162		(501)	GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG		
gp160.modSF162.delV2		(478)	-----GCC-----GG-----		
gp160.modSF162.delV1V2		(379)	-----GCC-----GG-----		
gp140.modSF162		(501)	GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG		
gp140.mut.modSF162		(501)	GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG		
gp140.mut7.modSF162		(501)	GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG		
gp140.mut8.modSF162		(501)	GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG		
gp120.modSF162		(501)	GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG		
Consensus		(501)	GAAGGAGTACGCCCTGTTCTACAAGCTGGACGTGGTGCCCATCGACAACG	551	600
gp160.modSF162		(551)	ACAACACCAGCTACAAGCTGATCAACTGCAACACCAGCGTGATCACCAG		
gp160.modSF162.delV2		(492)	-----CAAGCTGATCAACTGCAACACCAGCGTGATCACCAG		
gp160.modSF162.delV1V2		(384)	-----CAACTGCCAGACCAGCGTGATCACCAG		
gp140.modSF162		(551)	ACAACACCAGCTACAAGCTGATCAACTGCAACACCAGCGTGATCACCAG		
gp140.mut.modSF162		(551)	ACAACACCAGCTACAAGCTGATCAACTGCAACACCAGCGTGATCACCAG		

gp140.mut7.modSF162	(551)	ACAACACCAGCTACAAGCTGATCAACTGCAACACCAGCGTGATCACCAG
gp140.mut8.modSF162	(551)	ACAACACCAGCTACAAGCTGATCAACTGCAACACCAGCGTGATCACCAG
gp120.modSF162	(551)	ACAACACCAGCTACAAGCTGATCAACTGCAACACCAGCGTGATCACCAG
Consensus	(551)	ACAACACCAGCTACAAGCTGATCAACTGCAACACCAGCGTGATCACCAG
gp160.modSF162	(601)	GCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
gp160.modSF162.delV2	(520)	GCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
gp160.modSF162.delV1V2	(412)	GCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
gp140.modSF162	(601)	GCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
gp140.mut.modSF162	(601)	GCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
gp140.mut7.modSF162	(601)	GCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
gp140.mut8.modSF162	(601)	GCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
gp120.modSF162	(601)	GCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
Consensus	(601)	GCCTGCCCCAAGGTGAGCTTCGAGCCCATCCCCATCCACTACTGCGCCCC
gp160.modSF162	(651)	CGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCG
gp160.modSF162.delV2	(570)	CGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCG
gp160.modSF162.delV1V2	(462)	CGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCG
gp140.modSF162	(651)	CGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCG
gp140.mut.modSF162	(651)	CGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCG
gp140.mut7.modSF162	(651)	CGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCG
gp140.mut8.modSF162	(651)	CGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCG
gp120.modSF162	(651)	CGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCG
Consensus	(651)	CGCCGGCTTCGCCATCCTGAAGTGCAACGACAAGAAGTTCAACGGCAGCG
gp160.modSF162	(701)	GCCCCTGCACCAACGTGAGCACCCTGCAGTGCACCCACGGCATCCGCCCC
gp160.modSF162.delV2	(620)	GCCCCTGCACCAACGTGAGCACCCTGCAGTGCACCCACGGCATCCGCCCC
gp160.modSF162.delV1V2	(512)	GCCCCTGCACCAACGTGAGCACCCTGCAGTGCACCCACGGCATCCGCCCC
gp140.modSF162	(701)	GCCCCTGCACCAACGTGAGCACCCTGCAGTGCACCCACGGCATCCGCCCC
gp140.mut.modSF162	(701)	GCCCCTGCACCAACGTGAGCACCCTGCAGTGCACCCACGGCATCCGCCCC
gp140.mut7.modSF162	(701)	GCCCCTGCACCAACGTGAGCACCCTGCAGTGCACCCACGGCATCCGCCCC
gp140.mut8.modSF162	(701)	GCCCCTGCACCAACGTGAGCACCCTGCAGTGCACCCACGGCATCCGCCCC
gp120.modSF162	(701)	GCCCCTGCACCAACGTGAGCACCCTGCAGTGCACCCACGGCATCCGCCCC
Consensus	(701)	GCCCCTGCACCAACGTGAGCACCCTGCAGTGCACCCACGGCATCCGCCCC
gp160.modSF162	(751)	GTGGTGAGCACCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT
gp160.modSF162.delV2	(670)	GTGGTGAGCACCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT
gp160.modSF162.delV1V2	(562)	GTGGTGAGCACCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT
gp140.modSF162	(751)	GTGGTGAGCACCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT
gp140.mut.modSF162	(751)	GTGGTGAGCACCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT
gp140.mut7.modSF162	(751)	GTGGTGAGCACCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT
gp140.mut8.modSF162	(751)	GTGGTGAGCACCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT
gp120.modSF162	(751)	GTGGTGAGCACCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT
Consensus	(751)	GTGGTGAGCACCAGCTGCTGCTGAACGGCAGCCTGGCCGAGGAGGGCGT
gp160.modSF162	(801)	GGTGATCCGCAGCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGC
gp160.modSF162.delV2	(720)	GGTGATCCGCAGCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGC
gp160.modSF162.delV1V2	(612)	GGTGATCCGCAGCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGC
gp140.modSF162	(801)	GGTGATCCGCAGCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGC
gp140.mut.modSF162	(801)	GGTGATCCGCAGCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGC
gp140.mut7.modSF162	(801)	GGTGATCCGCAGCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGC
gp140.mut8.modSF162	(801)	GGTGATCCGCAGCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGC
gp120.modSF162	(801)	GGTGATCCGCAGCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGC
Consensus	(801)	GGTGATCCGCAGCGAGAACTTCACCGACAACGCCAAGACCATCATCGTGC
gp160.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCAACAACAACACC
gp160.modSF162.delV2	(770)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCAACAACAACACC
gp160.modSF162.delV1V2	(662)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCAACAACAACACC

FIG. 4A (Sheet 3/9)

gp140.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCAACAACAACACC	
gp140.mut.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCAACAACAACACC	
gp140.mut7.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCAACAACAACACC	
gp140.mut8.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCAACAACAACACC	
gp120.modSF162	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCAACAACAACACC	
Consensus	(851)	AGCTGAAGGAGAGCGTGGAGATCAACTGCACCCGCCCCAACAACAACACC	950
gp160.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGA	
gp160.modSF162.delV2	(820)	CGCAAGAGCATCACCATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGA	
gp160.modSF162.delV1V2	(712)	CGCAAGAGCATCACCATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGA	
gp140.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGA	
gp140.mut.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGA	
gp140.mut7.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGA	
gp140.mut8.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGA	
gp120.modSF162	(901)	CGCAAGAGCATCACCATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGA	
Consensus	(901)	CGCAAGAGCATCACCATCGGCCCCGGCCGCGCCTTCTACGCCACCGGCGA	1000
gp160.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCGGCGAGAAGT	
gp160.modSF162.delV2	(870)	CATCATCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCGGCGAGAAGT	
gp160.modSF162.delV1V2	(762)	CATCATCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCGGCGAGAAGT	
gp140.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCGGCGAGAAGT	
gp140.mut.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCGGCGAGAAGT	
gp140.mut7.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCGGCGAGAAGT	
gp140.mut8.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCGGCGAGAAGT	
gp120.modSF162	(951)	CATCATCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCGGCGAGAAGT	
Consensus	(951)	CATCATCGGCGACATCCGCCAGGCCCCACTGCAACATCAGCGGCGAGAAGT	1001
gp160.modSF162	(1001)	GGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCCCAGTTCGGC	
gp160.modSF162.delV2	(920)	GGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCCCAGTTCGGC	
gp160.modSF162.delV1V2	(812)	GGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCCCAGTTCGGC	
gp140.modSF162	(1001)	GGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCCCAGTTCGGC	
gp140.mut.modSF162	(1001)	GGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCCCAGTTCGGC	
gp140.mut7.modSF162	(1001)	GGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCCCAGTTCGGC	
gp140.mut8.modSF162	(1001)	GGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCCCAGTTCGGC	
gp120.modSF162	(1001)	GGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCCCAGTTCGGC	
Consensus	(1001)	GGAACAACACCCTGAAGCAGATCGTGACCAAGCTGCAGGCCCCAGTTCGGC	1051
gp160.modSF162	(1051)	AACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGT	
gp160.modSF162.delV2	(970)	AACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGT	
gp160.modSF162.delV1V2	(862)	AACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGT	
gp140.modSF162	(1051)	AACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGT	
gp140.mut.modSF162	(1051)	AACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGT	
gp140.mut7.modSF162	(1051)	AACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGT	
gp140.mut8.modSF162	(1051)	AACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGT	
gp120.modSF162	(1051)	AACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGT	
Consensus	(1051)	AACAAGACCATCGTGTTCAAGCAGAGCAGCGGCGGCGACCCCGAGATCGT	1101
gp160.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp160.modSF162.delV2	(1020)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp160.modSF162.delV1V2	(912)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp140.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp140.mut.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp140.mut7.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp140.mut8.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
gp120.modSF162	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	
Consensus	(1101)	GATGCACAGCTTCAACTGCGGCGGCGAGTTCTTCTACTGCAACAGCACCC	1151
gp160.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCCAACAACACCAAC	1200

gp160.modSF162.delV2	(1070)	AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCCAACAAACACCAAC
gp160.modSF162.delV1V2	(962)	AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCCAACAAACACCAAC
gp140.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCCAACAAACACCAAC
gp140.mut.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCCAACAAACACCAAC
gp140.mut7.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCCAACAAACACCAAC
gp140.mut8.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCCAACAAACACCAAC
gp120.modSF162	(1151)	AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCCAACAAACACCAAC
Consensus	(1151)	AGCTGTTCAACAGCACCTGGAACAACACCATCGGCCCCAACAAACACCAAC 1201 1250
gp160.modSF162	(1201)	GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA
gp160.modSF162.delV2	(1120)	GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA
gp160.modSF162.delV1V2	(1012)	GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA
gp140.modSF162	(1201)	GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA
gp140.mut.modSF162	(1201)	GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA
gp140.mut7.modSF162	(1201)	GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA
gp140.mut8.modSF162	(1201)	GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA
gp120.modSF162	(1201)	GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA
Consensus	(1201)	GGCACCATCACCTGCCCTGCCGCATCAAGCAGATCATCAACCGCTGGCA 1251 1300
gp160.modSF162	(1251)	GGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCGCT
gp160.modSF162.delV2	(1170)	GGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCGCT
gp160.modSF162.delV1V2	(1062)	GGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCGCT
gp140.modSF162	(1251)	GGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCGCT
gp140.mut.modSF162	(1251)	GGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCGCT
gp140.mut7.modSF162	(1251)	GGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCGCT
gp140.mut8.modSF162	(1251)	GGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCGCT
gp120.modSF162	(1251)	GGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCGCT
Consensus	(1251)	GGAGGTGGGCAAGGCCATGTACGCCCCCCCCATCCGCGGCCAGATCCGCT 1301 1350
gp160.modSF162	(1301)	GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG
gp160.modSF162.delV2	(1220)	GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG
gp160.modSF162.delV1V2	(1112)	GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG
gp140.modSF162	(1301)	GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG
gp140.mut.modSF162	(1301)	GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG
gp140.mut7.modSF162	(1301)	GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG
gp140.mut8.modSF162	(1301)	GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG
gp120.modSF162	(1301)	GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG
Consensus	(1301)	GCAGCAGCAACATCACCGGCCTGCTGCTGACCCGCGACGGCGGCAAGGAG 1351 1400
gp160.modSF162	(1351)	ATCAGCAACACCACCGAGATCTTCCGCCCGGGCGGCGGCGACATGCGCGA
gp160.modSF162.delV2	(1270)	ATCAGCAACACCACCGAGATCTTCCGCCCGGGCGGCGGCGACATGCGCGA
gp160.modSF162.delV1V2	(1162)	ATCAGCAACACCACCGAGATCTTCCGCCCGGGCGGCGGCGACATGCGCGA
gp140.modSF162	(1351)	ATCAGCAACACCACCGAGATCTTCCGCCCGGGCGGCGGCGACATGCGCGA
gp140.mut.modSF162	(1351)	ATCAGCAACACCACCGAGATCTTCCGCCCGGGCGGCGGCGACATGCGCGA
gp140.mut7.modSF162	(1351)	ATCAGCAACACCACCGAGATCTTCCGCCCGGGCGGCGGCGACATGCGCGA
gp140.mut8.modSF162	(1351)	ATCAGCAACACCACCGAGATCTTCCGCCCGGGCGGCGGCGACATGCGCGA
gp120.modSF162	(1351)	ATCAGCAACACCACCGAGATCTTCCGCCCGGGCGGCGGCGACATGCGCGA
Consensus	(1351)	ATCAGCAACACCACCGAGATCTTCCGCCCGGGCGGCGGCGACATGCGCGA 1401 1450
gp160.modSF162	(1401)	CAACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCC
gp160.modSF162.delV2	(1320)	CAACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCC
gp160.modSF162.delV1V2	(1212)	CAACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCC
gp140.modSF162	(1401)	CAACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCC
gp140.mut.modSF162	(1401)	CAACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCC
gp140.mut7.modSF162	(1401)	CAACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCC
gp140.mut8.modSF162	(1401)	CAACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCC
gp120.modSF162	(1401)	CAACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCC
Consensus	(1401)	CAACTGGCGCAGCGAGCTGTACAAGTACAAGGTGGTGAAGATCGAGCCCC

FIG 6 A (Sheet 5/9)

		1451		1500
gp160.modSF162	(1451)	TGGGCGTGGCCCCACCAAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG		
gp160.modSF162.delV2	(1370)	TGGGCGTGGCCCCACCAAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG		
gp160.modSF162.delV1V2	(1262)	TGGGCGTGGCCCCACCAAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG		
gp140.modSF162	(1451)	TGGGCGTGGCCCCACCAAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG		
gp140.mut.modSF162	(1451)	TGGGCGTGGCCCCACCAAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG		
gp140.mut7.modSF162	(1451)	TGGGCGTGGCCCCACCAAGGCCATCAGCAGCGTGGTGCAGAGCGAGAAG		
gp140.mut8.modSF162	(1451)	TGGGCGTGGCCCCACCATCGCCATCAGCAGCGTGGTGCAGAGCGAGAAG		
gp120.modSF162	(1451)	TGGGCGTGGCCCCACCAAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG		
Consensus	(1451)	TGGGCGTGGCCCCACCAAGGCCAAGCGCCGCGTGGTGCAGCGCGAGAAG		
		1501		1550
gp160.modSF162	(1501)	CGCGCCGTGACCCTGGGCGCCATGTTCTTGGGCTTCCTGGGCGCCGCCGG		
gp160.modSF162.delV2	(1420)	CGCGCCGTGACCCTGGGCGCCATGTTCTTGGGCTTCCTGGGCGCCGCCGG		
gp160.modSF162.delV1V2	(1312)	CGCGCCGTGACCCTGGGCGCCATGTTCTTGGGCTTCCTGGGCGCCGCCGG		
gp140.modSF162	(1501)	CGCGCCGTGACCCTGGGCGCCATGTTCTTGGGCTTCCTGGGCGCCGCCGG		
gp140.mut.modSF162	(1501)	AGCGCCGTGACCCTGGGCGCCATGTTCTTGGGCTTCCTGGGCGCCGCCGG		
gp140.mut7.modSF162	(1501)	AGCGCCGTGACCCTGGGCGCCATGTTCTTGGGCTTCCTGGGCGCCGCCGG		
gp140.mut8.modSF162	(1501)	AGCGCCGTGACCCTGGGCGCCATGTTCTTGGGCTTCCTGGGCGCCGCCGG		
gp120.modSF162	(1501)	CGC----TAACTCGAG-----		
Consensus	(1501)	CGCGCCGTGACCCTGGGCGCCATGTTCTTGGGCTTCCTGGGCGCCGCCGG		
		1551		1600
gp160.modSF162	(1551)	CAGCACCATGGGCGCCCGCAGCCTGACCCTGACCGTGCAGGCCCGCCAGC		
gp160.modSF162.delV2	(1470)	CAGCACCATGGGCGCCCGCAGCCTGACCCTGACCGTGCAGGCCCGCCAGC		
gp160.modSF162.delV1V2	(1362)	CAGCACCATGGGCGCCCGCAGCCTGACCCTGACCGTGCAGGCCCGCCAGC		
gp140.modSF162	(1551)	CAGCACCATGGGCGCCCGCAGCCTGACCCTGACCGTGCAGGCCCGCCAGC		
gp140.mut.modSF162	(1551)	CAGCACCATGGGCGCCCGCAGCCTGACCCTGACCGTGCAGGCCCGCCAGC		
gp140.mut7.modSF162	(1551)	CAGCACCATGGGCGCCCGCAGCCTGACCCTGACCGTGCAGGCCCGCCAGC		
gp140.mut8.modSF162	(1551)	CAGCACCATGGGCGCCCGCAGCCTGACCCTGACCGTGCAGGCCCGCCAGC		
gp120.modSF162	(1513)	-----		
Consensus	(1551)	CAGCACCATGGGCGCCCGCAGCCTGACCCTGACCGTGCAGGCCCGCCAGC		
		1601		1650
gp160.modSF162	(1601)	TGCTGAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAG		
gp160.modSF162.delV2	(1520)	TGCTGAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAG		
gp160.modSF162.delV1V2	(1412)	TGCTGAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAG		
gp140.modSF162	(1601)	TGCTGAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAG		
gp140.mut.modSF162	(1601)	TGCTGAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAG		
gp140.mut7.modSF162	(1601)	TGCTGAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAG		
gp140.mut8.modSF162	(1601)	TGCTGAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAG		
gp120.modSF162	(1513)	-----		
Consensus	(1601)	TGCTGAGCGGCATCGTGCAGCAGCAGAACAACCTGCTGCGCGCCATCGAG		
		1651		1700
gp160.modSF162	(1651)	GCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCA		
gp160.modSF162.delV2	(1570)	GCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCA		
gp160.modSF162.delV1V2	(1462)	GCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCA		
gp140.modSF162	(1651)	GCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCA		
gp140.mut.modSF162	(1651)	GCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCA		
gp140.mut7.modSF162	(1651)	GCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCA		
gp140.mut8.modSF162	(1651)	GCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCA		
gp120.modSF162	(1513)	-----		
Consensus	(1651)	GCCCAGCAGCACCTGCTGCAGCTGACCGTGTGGGGCATCAAGCAGCTGCA		
		1701		1750
gp160.modSF162	(1701)	GGCCCCGCTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG		
gp160.modSF162.delV2	(1620)	GGCCCCGCTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG		
gp160.modSF162.delV1V2	(1512)	GGCCCCGCTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG		
gp140.modSF162	(1701)	GGCCCCGCTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG		
gp140.mut.modSF162	(1701)	GGCCCCGCTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG		
gp140.mut7.modSF162	(1701)	GGCCCCGCTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG		
gp140.mut8.modSF162	(1701)	GGCCCCGCTGCTGGCCGTGGAGCGCTACCTGAAGGACCAGCAGCTGCTGG		

FIG. 6A (Sheet 6/9)

gp140.mut7.modSF162	(2001)	GTGGCTGTGGTACATCTAACTCGAG-----	
gp140.mut8.modSF162	(2001)	GTGGCTGTGGTACATCTAACTCGAG-----	
gp120.modSF162	(1513)	-----	
Consensus	(2001)	GTGGCTGTGGTACATCTAACTCGAG	2100
		2051	
gp160.modSF162	(2051)	GCCTGCGCATCGTGTTCACCGTGCTGAGCATCGTGAACCGCGTGCGCCAG	
gp160.modSF162.delV2	(1970)	GCCTGCGCATCGTGTTCACCGTGCTGAGCATCGTGAACCGCGTGCGCCAG	
gp160.modSF162.delV1V2	(1862)	GCCTGCGCATCGTGTTCACCGTGCTGAGCATCGTGAACCGCGTGCGCCAG	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2051)		2150
		2101	
gp160.modSF162	(2101)	GGCTACAGCCCCCTGAGCTTCCAGACCCGCTTCCCCGCCCCCGCGGCC	
gp160.modSF162.delV2	(2020)	GGCTACAGCCCCCTGAGCTTCCAGACCCGCTTCCCCGCCCCCGCGGCC	
gp160.modSF162.delV1V2	(1912)	GGCTACAGCCCCCTGAGCTTCCAGACCCGCTTCCCCGCCCCCGCGGCC	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2101)		2200
		2151	
gp160.modSF162	(2151)	CGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACC	
gp160.modSF162.delV2	(2070)	CGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACC	
gp160.modSF162.delV1V2	(1962)	CGACCGCCCCGAGGGCATCGAGGAGGAGGGCGGCGAGCGCGACCGCGACC	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2151)		2250
		2201	
gp160.modSF162	(2201)	GCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGGGACGACCTG	
gp160.modSF162.delV2	(2120)	GCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGGGACGACCTG	
gp160.modSF162.delV1V2	(2012)	GCAGCAGCCCCCTGGTGCACGGCCTGCTGGCCCTGATCTGGGACGACCTG	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2201)		2300
		2251	
gp160.modSF162	(2251)	CGCAGCCTGTGCCTGTTCAGCTACCACCGCCTGCGCGACCTGATCCTGAT	
gp160.modSF162.delV2	(2170)	CGCAGCCTGTGCCTGTTCAGCTACCACCGCCTGCGCGACCTGATCCTGAT	
gp160.modSF162.delV1V2	(2062)	CGCAGCCTGTGCCTGTTCAGCTACCACCGCCTGCGCGACCTGATCCTGAT	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2251)		2350
		2301	
gp160.modSF162	(2301)	CGCCGCCCCGATCGTGGAGCTGCTGGGCCGCCGCGGCTGGGAGGCCCTGA	
gp160.modSF162.delV2	(2220)	CGCCGCCCCGATCGTGGAGCTGCTGGGCCGCCGCGGCTGGGAGGCCCTGA	
gp160.modSF162.delV1V2	(2112)	CGCCGCCCCGATCGTGGAGCTGCTGGGCCGCCGCGGCTGGGAGGCCCTGA	

FIG. 66A (Sheet 8/9)

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gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2301)		
		2351	2400
gp160.modSF162	(2351)	AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC	
gp160.modSF162.delV2	(2270)	AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC	
gp160.modSF162.delV1V2	(2162)	AGTACTGGGGCAACCTGCTGCAGTACTGGATCCAGGAGCTGAAGAACAGC	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2351)		
		2401	2450
gp160.modSF162	(2401)	GCCGTGAGCCTGTTTCGACGCCATCGCCATCGCCGTGGCCGAGGGCACC GA	
gp160.modSF162.delV2	(2320)	GCCGTGAGCCTGTTTCGACGCCATCGCCATCGCCGTGGCCGAGGGCACC GA	
gp160.modSF162.delV1V2	(2212)	GCCGTGAGCCTGTTTCGACGCCATCGCCATCGCCGTGGCCGAGGGCACC GA	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2401)		
		2451	2500
gp160.modSF162	(2451)	CCGCATCATCGAGGTGGCCCAGCGCATCGGCCGCGCCTTCCTGCACATCC	
gp160.modSF162.delV2	(2370)	CCGCATCATCGAGGTGGCCCAGCGCATCGGCCGCGCCTTCCTGCACATCC	
gp160.modSF162.delV1V2	(2262)	CCGCATCATCGAGGTGGCCCAGCGCATCGGCCGCGCCTTCCTGCACATCC	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2451)		
		2501	2547
gp160.modSF162	(2501)	CCCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCTGTAACTCGAG	
gp160.modSF162.delV2	(2420)	CCCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCTGTAACTCGAG	
gp160.modSF162.delV1V2	(2312)	CCCGCCGCATCCGCCAGGGCTTCGAGCGCGCCCTGCTGTAACTCGAG	
gp140.modSF162	(2026)	-----	
gp140.mut.modSF162	(2026)	-----	
gp140.mut7.modSF162	(2026)	-----	
gp140.mut8.modSF162	(2026)	-----	
gp120.modSF162	(1513)	-----	
Consensus	(2501)		

			Start of tPA	
		1	↓	40
gp160	(1)	GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT		
gp160 del V1	(1)	GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT		
gp160 del V2	(1)	GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT		
gp160 del V1-2	(1)	GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT		
gp 160 del 128-194	(1)	GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT		
gp140TM	(1)	GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT		
gp140	(1)	GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT		
gp140mut	(1)	GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT		
gp120	(1)	GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT		
Consensus	(1)	GAATTGCGCCACCATGGATGCAATGAAGAGAGGGGCTCTGCT		
		41		80
gp160	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp160 del V1	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp160 del V2	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp160 del V1-2	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp 160 del 128-194	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp140TM	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp140	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp140mut	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
gp120	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
Consensus	(41)	GTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCAG		
		end of tPA	↓	
		81		120
gp160	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTG		
gp160 del V1	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTG		
gp160 del V2	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTG		
gp160 del V1-2	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTG		
gp 160 del 128-194	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTG		
gp140TM	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTG		
gp140	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTG		
gp140mut	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTG		
gp120	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTG		
Consensus	(81)	CGCCACCACCGTGCTGTGGGTGACCGTGTACTACGGCGTG		
		121		160
gp160	(121)	CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCA		
gp160 del V1	(121)	CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCA		
gp160 del V2	(121)	CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCA		
gp160 del V1-2	(121)	CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCA		
gp 160 del 128-194	(121)	CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCA		
gp140TM	(121)	CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCA		
gp140	(121)	CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCA		
gp140mut	(121)	CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCA		
gp120	(121)	CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCA		
Consensus	(121)	CCCGTGTGGAAGGAGGCCACCACCACCTGTTCTGCGCCA		

FIG. 6B (Sheet 1/14)

		161	200
gp160	(161)	GCGAAGCCCAAGGCTTACAAGGCCGAGGCCACAGAAGCTGTG	
gp160 del V1	(161)	GCGAAGCCCAAGGCTTACAAGGCCGAGGCCACAGAAGCTGTG	
gp160 del V2	(161)	GCGAAGCCCAAGGCTTACAAGGCCGAGGCCACAGAAGCTGTG	
gp160 del V1-2	(161)	GCGAAGCCCAAGGCTTACAAGGCCGAGGCCACAGAAGCTGTG	
gp 160 del 128-194	(161)	GCGAAGCCCAAGGCTTACAAGGCCGAGGCCACAGAAGCTGTG	
gp140TM	(161)	GCGAAGCCCAAGGCTTACAAGGCCGAGGCCACAGAAGCTGTG	
gp140	(161)	GCGAAGCCCAAGGCTTACAAGGCCGAGGCCACAGAAGCTGTG	
gp140mut	(161)	GCGAAGCCCAAGGCTTACAAGGCCGAGGCCACAGAAGCTGTG	
gp120	(161)	GCGAAGCCCAAGGCTTACAAGGCCGAGGCCACAGAAGCTGTG	
Consensus	(161)	GCGACGCCAAGGCTTACAAGGCCGAGGCCACAGAAGCTGTG	
		201	240
gp160	(201)	GGCCACCCACGCGCTGCGTGCCACCCAGCCCCAACCCCCAG	
gp160 del V1	(201)	GGCCACCCACGCGCTGCGTGCCACCCAGCCCCAACCCCCAG	
gp160 del V2	(201)	GGCCACCCACGCGCTGCGTGCCACCCAGCCCCAACCCCCAG	
gp160 del V1-2	(201)	GGCCACCCACGCGCTGCGTGCCACCCAGCCCCAACCCCCAG	
gp 160 del 128-194	(201)	GGCCACCCACGCGCTGCGTGCCACCCAGCCCCAACCCCCAG	
gp140TM	(201)	GGCCACCCACGCGCTGCGTGCCACCCAGCCCCAACCCCCAG	
gp140	(201)	GGCCACCCACGCGCTGCGTGCCACCCAGCCCCAACCCCCAG	
gp140mut	(201)	GGCCACCCACGCGCTGCGTGCCACCCAGCCCCAACCCCCAG	
gp120	(201)	GGCCACCCACGCGCTGCGTGCCACCCAGCCCCAACCCCCAG	
Consensus	(201)	GGCCACCCACGCGCTGCGTGCCACCCAGCCCCAACCCCCAG	
		241	280
gp160	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp160 del V1	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp160 del V2	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp160 del V1-2	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp 160 del 128-194	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp140TM	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp140	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp140mut	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
gp120	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
Consensus	(241)	GAGGTGAACCTGACCAACGTGACCGAGAACTTCAACATGT	
		281	320
gp160	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp160 del V1	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp160 del V2	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp160 del V1-2	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp 160 del 128-194	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp140TM	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp140	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp140mut	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
gp120	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
Consensus	(281)	GGAAGAACAACATGGTGGAGCAGATGCATGAGGACATCAT	
		321	360
gp160	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp160 del V1	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp160 del V2	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp160 del V1-2	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp 160 del 128-194	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp140TM	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp140	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp140mut	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
gp120	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	
Consensus	(321)	CAGCCTGTGGGACCAGAGCCTGAAGCCCTGCGTGAAGCTG	

FIG. 66 B (Sheet 2/14)

		361	400
gp160	(361)	ACCCCTGTCGCTGACCCCTGAACCTGCACCGACAAGCTGA	
gp160 del V1	(361)	ACCCCTGTCGCTGACCCCTGAACCTGCACCGACAAGCTG	G
gp160 del V2	(361)	ACCCCTGTCGCTGACCCCTGAACCTGCACCGACAAGCTGA	
gp160 del V1-2	(361)	GGC-----	
gp 160 del 128-194	(361)	ACCCCTGTCGCTGACCCCTGAACCTGCACCGACAAGCTGA	
gp140TM	(361)	ACCCCTGTCGCTGACCCCTGAACCTGCACCGACAAGCTGA	
gp140	(361)	ACCCCTGTCGCTGACCCCTGAACCTGCACCGACAAGCTGA	
gp140mut	(361)	ACCCCTGTCGCTGACCCCTGAACCTGCACCGACAAGCTGA	
gp120	(361)	ACCCCTGTCGCTGACCCCTGAACCTGCACCGACAAGCTGA	
Consensus	(361)	ACCCCTGTCGCTGACCCCTGAACCTGCACCGACAAGCTGA	
		401	440
gp160	(401)	CCGGCAGCACCACCGGCACCAACAGCACCAGCGGCACCAA	
gp160 del V1	(401)	CCGGCAGCACCACCGGCACCAACAGCACCAGCGGCACCAA	
gp160 del V2	(401)	CCGGCAGCACCACCGGCACCAACAGCACCAGCGGCACCAA	
gp160 del V1-2	(364)	-----	
gp 160 del 128-194	(385)	-----	
gp140TM	(401)	CCGGCAGCACCACCGGCACCAACAGCACCAGCGGCACCAA	
gp140	(401)	CCGGCAGCACCACCGGCACCAACAGCACCAGCGGCACCAA	
gp140mut	(401)	CCGGCAGCACCACCGGCACCAACAGCACCAGCGGCACCAA	
gp120	(401)	CCGGCAGCACCACCGGCACCAACAGCACCAGCGGCACCAA	
Consensus	(401)	CCGGCAGCACCACCGGCACCAACAGCACCAGCGGCACCAA	
		441	480
gp160	(441)	CAGCACCAGCGGCACCAACAGCACCAGCAGCAACAGCACC	
gp160 del V1	(409)	-----	
gp160 del V2	(441)	CAGCACCAGCGGCACCAACAGCACCAGCAGCAACAGCACC	
gp160 del V1-2	(364)	-----	
gp 160 del 128-194	(385)	-----	
gp140TM	(441)	CAGCACCAGCGGCACCAACAGCACCAGCAGCAACAGCACC	
gp140	(441)	CAGCACCAGCGGCACCAACAGCACCAGCAGCAACAGCACC	
gp140mut	(441)	CAGCACCAGCGGCACCAACAGCACCAGCAGCAACAGCACC	
gp120	(441)	CAGCACCAGCGGCACCAACAGCACCAGCAGCAACAGCACC	
Consensus	(441)	CAGCACCAGCGGCACCAACAGCACCAGCAGCAACAGCACC	
		481	520
gp160	(481)	GACAGCTGGGAGAAGATGCCCGAGGGGCGAGATCAAGAACT	
gp160 del V1	(409)	-----GGCGAGATCAAGAACT	
gp160 del V2	(481)	GACAGCTGGGAGAAGATGCCCGAGGGGCGAGATCAAGAACT	
gp160 del V1-2	(364)	-----	
gp 160 del 128-194	(385)	-----	
gp140TM	(481)	GACAGCTGGGAGAAGATGCCCGAGGGGCGAGATCAAGAACT	
gp140	(481)	GACAGCTGGGAGAAGATGCCCGAGGGGCGAGATCAAGAACT	
gp140mut	(481)	GACAGCTGGGAGAAGATGCCCGAGGGGCGAGATCAAGAACT	
gp120	(481)	GACAGCTGGGAGAAGATGCCCGAGGGGCGAGATCAAGAACT	
Consensus	(481)	GACAGCTGGGAGAAGATGCCCGAGGGGCGAGATCAAGAACT	
		521	560
gp160	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
gp160 del V1	(425)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
gp160 del V2	(521)	GCAGCTTCAACATCGGGGCGCGG-----	
gp160 del V1-2	(364)	-----	
gp 160 del 128-194	(385)	-----	
gp140TM	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
gp140	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
gp140mut	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
gp120	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	
Consensus	(521)	GCAGCTTCAACATCACCACCAGCGTGCGCGACAAGGTGCA	

		561	600
gp160	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGAAGTGGTGCCC	
gp160 del V1	(465)	GAAGGAGTACAGCCTGTTCTACAAGCTGGAAGTGGTGCCC	
gp160 del V2	(544)	-----	
gp160 del V1-2	(364)	-----	
gp 160 del 128-194	(385)	-----	
gp140TM	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGAAGTGGTGCCC	
gp140	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGAAGTGGTGCCC	
gp140mut	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGAAGTGGTGCCC	
gp120	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGAAGTGGTGCCC	
Consensus	(561)	GAAGGAGTACAGCCTGTTCTACAAGCTGGAAGTGGTGCCC	
		601	640
gp160	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
gp160 del V1	(505)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
gp160 del V2	(544)	-----GCGCTGATCAACTGCA	
gp160 del V1-2	(364)	-----	
gp 160 del 128-194	(385)	-----AACTGCG	
gp140TM	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
gp140	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
gp140mut	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
gp120	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
Consensus	(601)	ATCGACAACGACAACGCCAGCTACCGCCTGATCAACTGCA	
		641	680
gp160	(641)	ACACGAGCGTGATCACCCAGGCTGCCCCAAGGTGAGCTT	
gp160 del V1	(545)	ACACGAGCGTGATCACCCAGGCTGCCCCAAGGTGAGCTT	
gp160 del V2	(560)	ACACGAGCGTGATCACCCAGGCTGCCCCAAGGTGAGCTT	
gp160 del V1-2	(364)	-----GAGGCTGCCCCAAGGTGAGCTT	
gp 160 del 128-194	(392)	AGACGAGCGTGATCACCCAGGCTGCCCCAAGGTGAGCTT	
gp140TM	(641)	ACACGAGCGTGATCACCCAGGCTGCCCCAAGGTGAGCTT	
gp140	(641)	ACACGAGCGTGATCACCCAGGCTGCCCCAAGGTGAGCTT	
gp140mut	(641)	ACACGAGCGTGATCACCCAGGCTGCCCCAAGGTGAGCTT	
gp120	(641)	ACACGAGCGTGATCACCCAGGCTGCCCCAAGGTGAGCTT	
Consensus	(641)	ACACGAGCGTGATCACCCAGGCTGCCCCAAGGTGAGCTT	
		681	720
gp160	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCGCGGCTTC	
gp160 del V1	(585)	CGAGCCCATCCCCATCCACTACTGCGCCCCGCGGCTTC	
gp160 del V2	(600)	CGAGCCCATCCCCATCCACTACTGCGCCCCGCGGCTTC	
gp160 del V1-2	(387)	CGAGCCCATCCCCATCCACTACTGCGCCCCGCGGCTTC	
gp 160 del 128-194	(432)	CGAGCCCATCCCCATCCACTACTGCGCCCCGCGGCTTC	
gp140TM	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCGCGGCTTC	
gp140	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCGCGGCTTC	
gp140mut	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCGCGGCTTC	
gp120	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCGCGGCTTC	
Consensus	(681)	CGAGCCCATCCCCATCCACTACTGCGCCCCGCGGCTTC	
		721	760
gp160	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp160 del V1	(625)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp160 del V2	(640)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp160 del V1-2	(427)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp 160 del 128-194	(472)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp140TM	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp140	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp140mut	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
gp120	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	
Consensus	(721)	GCCATCCTGAAGTGCAAGGACAAGAAGTTCAACGGCACCG	

FIG 66 B (Sheet 4/14)

		761	800
gp160	(761)	SCCCCTGCAAGAACCTGAGGACCCCTGCACTGCACCCACGG	
gp160 del V1	(665)	SCCCCTGCAAGAACCTGAGGACCCCTGCACTGCACCCACGG	
gp160 del V2	(680)	SCCCCTGCAAGAACCTGAGGACCCCTGCACTGCACCCACGG	
gp160 del V1-2	(467)	SCCCCTGCAAGAACCTGAGGACCCCTGCACTGCACCCACGG	
gp 160 del 128-194	(512)	SCCCCTGCAAGAACCTGAGGACCCCTGCACTGCACCCACGG	
gp140TM	(761)	SCCCCTGCAAGAACCTGAGGACCCCTGCACTGCACCCACGG	
gp140	(761)	SCCCCTGCAAGAACCTGAGGACCCCTGCACTGCACCCACGG	
gp140mut	(761)	SCCCCTGCAAGAACCTGAGGACCCCTGCACTGCACCCACGG	
gp120	(761)	SCCCCTGCAAGAACCTGAGGACCCCTGCACTGCACCCACGG	
Consensus	(761)	GCCCCGTGCAAGAACCTGAGGACCCCTGCACTGCACCCACGG	
		801	840
gp160	(801)	CATCCGCCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp160 del V1	(705)	CATCCGCCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp160 del V2	(720)	CATCCGCCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp160 del V1-2	(507)	CATCCGCCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp 160 del 128-194	(552)	CATCCGCCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp140TM	(801)	CATCCGCCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp140	(801)	CATCCGCCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp140mut	(801)	CATCCGCCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
gp120	(801)	CATCCGCCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
Consensus	(801)	CATCCGCCCCCGTGGTGAGCACCCAGCTGCTGCTGAACGGC	
		841	880
gp160	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp160 del V1	(745)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp160 del V2	(760)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp160 del V1-2	(547)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp 160 del 128-194	(592)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp140TM	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp140	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp140mut	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
gp120	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
Consensus	(841)	AGCCTGGCCGAGGAGGAGATCGTGCTGCGCTCCGAGAACT	
		881	920
gp160	(881)	TCACCGACAACGCCAAGACCATCATCGTGCAAGCTGAACGA	
gp160 del V1	(785)	TCACCGACAACGCCAAGACCATCATCGTGCAAGCTGAACGA	
gp160 del V2	(800)	TCACCGACAACGCCAAGACCATCATCGTGCAAGCTGAACGA	
gp160 del V1-2	(587)	TCACCGACAACGCCAAGACCATCATCGTGCAAGCTGAACGA	
gp 160 del 128-194	(632)	TCACCGACAACGCCAAGACCATCATCGTGCAAGCTGAACGA	
gp140TM	(881)	TCACCGACAACGCCAAGACCATCATCGTGCAAGCTGAACGA	
gp140	(881)	TCACCGACAACGCCAAGACCATCATCGTGCAAGCTGAACGA	
gp140mut	(881)	TCACCGACAACGCCAAGACCATCATCGTGCAAGCTGAACGA	
gp120	(881)	TCACCGACAACGCCAAGACCATCATCGTGCAAGCTGAACGA	
Consensus	(881)	TCACCGACAACGCCAAGACCATCATCGTGCAAGCTGAACGA	
		921	960
gp160	(921)	GTCCGTGGAGATCAACTGCATCCGCCCAACAACAACACG	
gp160 del V1	(825)	GTCCGTGGAGATCAACTGCATCCGCCCAACAACAACACG	
gp160 del V2	(840)	GTCCGTGGAGATCAACTGCATCCGCCCAACAACAACACG	
gp160 del V1-2	(627)	GTCCGTGGAGATCAACTGCATCCGCCCAACAACAACACG	
gp 160 del 128-194	(672)	GTCCGTGGAGATCAACTGCATCCGCCCAACAACAACACG	
gp140TM	(921)	GTCCGTGGAGATCAACTGCATCCGCCCAACAACAACACG	
gp140	(921)	GTCCGTGGAGATCAACTGCATCCGCCCAACAACAACACG	
gp140mut	(921)	GTCCGTGGAGATCAACTGCATCCGCCCAACAACAACACG	
gp120	(921)	GTCCGTGGAGATCAACTGCATCCGCCCAACAACAACACG	
Consensus	(921)	GTCCGTGGAGATCAACTGCATCCGCCCAACAACAACACG	

FIG.4B (Sheet 5/14)

		961	1000
gp160	(961)	CGTAAGAGCATCCAGATCGGGGGGGGGGGGGGGGGCTTCTACG	
gp160 del V1	(865)	CGTAAGAGCATCCAGATCGGGGGGGGGGGGGGGGGCTTCTACG	
gp160 del V2	(880)	CGTAAGAGCATCCAGATCGGGGGGGGGGGGGGGGGCTTCTACG	
gp160 del V1-2	(667)	CGTAAGAGCATCCAGATCGGGGGGGGGGGGGGGGGCTTCTACG	
gp 160 del 128-194	(712)	CGTAAGAGCATCCAGATCGGGGGGGGGGGGGGGGGCTTCTACG	
gp140TM	(961)	CGTAAGAGCATCCAGATCGGGGGGGGGGGGGGGGGCTTCTACG	
gp140	(961)	CGTAAGAGCATCCAGATCGGGGGGGGGGGGGGGGGCTTCTACG	
gp140mut	(961)	CGTAAGAGCATCCAGATCGGGGGGGGGGGGGGGGGCTTCTACG	
gp120	(961)	CGTAAGAGCATCCAGATCGGGGGGGGGGGGGGGGGCTTCTACG	
Consensus	(961)	CGTAAGAGCATCCAGATCGGGGGGGGGGGGGGGGGCTTCTACG	
		1001	1040
gp160	(1001)	CCACCGGCGACATCATCGGGGAGATCCGGCCAGGGCCACTG	
gp160 del V1	(905)	CCACCGGCGACATCATCGGGGAGATCCGGCCAGGGCCACTG	
gp160 del V2	(920)	CCACCGGCGACATCATCGGGGAGATCCGGCCAGGGCCACTG	
gp160 del V1-2	(707)	CCACCGGCGACATCATCGGGGAGATCCGGCCAGGGCCACTG	
gp 160 del 128-194	(752)	CCACCGGCGACATCATCGGGGAGATCCGGCCAGGGCCACTG	
gp140TM	(1001)	CCACCGGCGACATCATCGGGGAGATCCGGCCAGGGCCACTG	
gp140	(1001)	CCACCGGCGACATCATCGGGGAGATCCGGCCAGGGCCACTG	
gp140mut	(1001)	CCACCGGCGACATCATCGGGGAGATCCGGCCAGGGCCACTG	
gp120	(1001)	CCACCGGCGACATCATCGGGGAGATCCGGCCAGGGCCACTG	
Consensus	(1001)	CCACCGGCGACATCATCGGGGAGATCCGGCCAGGGCCACTG	
		1041	1080
gp160	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp160 del V1	(945)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp160 del V2	(960)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp160 del V1-2	(747)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp 160 del 128-194	(792)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp140TM	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp140	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp140mut	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
gp120	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
Consensus	(1041)	CAACATCAGCAAGGCCAACTGGACCAACACCCTCGAGCAG	
		1081	1120
gp160	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTGGGCAACAACAAGA	
gp160 del V1	(985)	ATCGTGGAGAAGCTGCGCGAGCAGTTGGGCAACAACAAGA	
gp160 del V2	(1000)	ATCGTGGAGAAGCTGCGCGAGCAGTTGGGCAACAACAAGA	
gp160 del V1-2	(787)	ATCGTGGAGAAGCTGCGCGAGCAGTTGGGCAACAACAAGA	
gp 160 del 128-194	(832)	ATCGTGGAGAAGCTGCGCGAGCAGTTGGGCAACAACAAGA	
gp140TM	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTGGGCAACAACAAGA	
gp140	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTGGGCAACAACAAGA	
gp140mut	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTGGGCAACAACAAGA	
gp120	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTGGGCAACAACAAGA	
Consensus	(1081)	ATCGTGGAGAAGCTGCGCGAGCAGTTGGGCAACAACAAGA	
		1121	1160
gp160	(1121)	CCATCATCTTCAACAGCAGCAGCGGGCGGGGACCCCGAGAT	
gp160 del V1	(1025)	CCATCATCTTCAACAGCAGCAGCGGGCGGGGACCCCGAGAT	
gp160 del V2	(1040)	CCATCATCTTCAACAGCAGCAGCGGGCGGGGACCCCGAGAT	
gp160 del V1-2	(827)	CCATCATCTTCAACAGCAGCAGCGGGCGGGGACCCCGAGAT	
gp 160 del 128-194	(872)	CCATCATCTTCAACAGCAGCAGCGGGCGGGGACCCCGAGAT	
gp140TM	(1121)	CCATCATCTTCAACAGCAGCAGCGGGCGGGGACCCCGAGAT	
gp140	(1121)	CCATCATCTTCAACAGCAGCAGCGGGCGGGGACCCCGAGAT	
gp140mut	(1121)	CCATCATCTTCAACAGCAGCAGCGGGCGGGGACCCCGAGAT	
gp120	(1121)	CCATCATCTTCAACAGCAGCAGCGGGCGGGGACCCCGAGAT	
Consensus	(1121)	CCATCATCTTCAACAGCAGCAGCGGGCGGGGACCCCGAGAT	

FIG. 4B (Sheet 6/14)

		1161	1200
gp160	(1161)	CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC	
gp160 del V1	(1065)	CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC	
gp160 del V2	(1080)	CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC	
gp160 del V1-2	(867)	CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC	
gp 160 del 128-194	(912)	CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC	
gp140TM	(1161)	CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC	
gp140	(1161)	CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC	
gp140mut	(1161)	CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC	
gp120	(1161)	CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC	
Consensus	(1161)	CGTGTTCACAGCTTCAACTGCGGCGGCGAGTTCTTCTAC	
		1201	1240
gp160	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA	
gp160 del V1	(1105)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA	
gp160 del V2	(1120)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA	
gp160 del V1-2	(907)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA	
gp 160 del 128-194	(952)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA	
gp140TM	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA	
gp140	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA	
gp140mut	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA	
gp120	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA	
Consensus	(1201)	TGCAACACCAGCCAGCTGTTCAACAGCACCTGGAACATCA	
		1241	1280
gp160	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT	
gp160 del V1	(1145)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT	
gp160 del V2	(1160)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT	
gp160 del V1-2	(947)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT	
gp 160 del 128-194	(992)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT	
gp140TM	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT	
gp140	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT	
gp140mut	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT	
gp120	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT	
Consensus	(1241)	CCGAGGAGGTGAACAAGACCAAGGAGAACGACACCATCAT	
		1281	1320
gp160	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG	
gp160 del V1	(1185)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG	
gp160 del V2	(1200)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG	
gp160 del V1-2	(987)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG	
gp 160 del 128-194	(1032)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG	
gp140TM	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG	
gp140	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG	
gp140mut	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG	
gp120	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG	
Consensus	(1281)	CCTGCCCTGCCGCATCCGCCAGATCATCAACATGTGGCAG	
		1321	1360
gp160	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC	
gp160 del V1	(1225)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC	
gp160 del V2	(1240)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC	
gp160 del V1-2	(1027)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC	
gp 160 del 128-194	(1072)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC	
gp140TM	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC	
gp140	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC	
gp140mut	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC	
gp120	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC	
Consensus	(1321)	GAGGTGGGCAAGGCCATGTACGCCCCCCCCCATCCGCGGCC	

FIG. 6 B (Sheet 7/14)

		1361	1400
gp160	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp160 del V1	(1265)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp160 del V2	(1280)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp160 del V1-2	(1067)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp 160 del 128-194	(1112)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp140TM	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp140	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp140mut	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
gp120	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
Consensus	(1361)	AGATCAAGTGCAGCAGCAATATTACCGGCCTGCTGCTGAC	
		1401	1440
gp160	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp160 del V1	(1305)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp160 del V2	(1320)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp160 del V1-2	(1107)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp 160 del 128-194	(1152)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp140TM	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp140	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp140mut	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
gp120	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
Consensus	(1401)	CCGCGACGGCGGCACCAACAACAACCGCACCAACGACACC	
		1441	1480
gp160	(1441)	GAGACCTTCGGCGCGCGCGCGCGCAACATGAAGGACAACCT	
gp160 del V1	(1345)	GAGACCTTCGGCGCGCGCGCGCGCAACATGAAGGACAACCT	
gp160 del V2	(1360)	GAGACCTTCGGCGCGCGCGCGCGCAACATGAAGGACAACCT	
gp160 del V1-2	(1147)	GAGACCTTCGGCGCGCGCGCGCGCAACATGAAGGACAACCT	
gp 160 del 128-194	(1192)	GAGACCTTCGGCGCGCGCGCGCGCAACATGAAGGACAACCT	
gp140TM	(1441)	GAGACCTTCGGCGCGCGCGCGCGCAACATGAAGGACAACCT	
gp140	(1441)	GAGACCTTCGGCGCGCGCGCGCGCAACATGAAGGACAACCT	
gp140mut	(1441)	GAGACCTTCGGCGCGCGCGCGCGCAACATGAAGGACAACCT	
gp120	(1441)	GAGACCTTCGGCGCGCGCGCGCGCAACATGAAGGACAACCT	
Consensus	(1441)	GAGACCTTCGGCGCGCGCGCGCGCAACATGAAGGACAACCT	
		1481	1520
gp160	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp160 del V1	(1385)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp160 del V2	(1400)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp160 del V1-2	(1187)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp 160 del 128-194	(1232)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp140TM	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp140	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp140mut	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
gp120	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
Consensus	(1481)	GGCGCAGCGAGCTGTACAAGTACAAGGTGGTGCGCATCGA	
		1521	1560
gp160	(1521)	GGCGCTGGGCGTGGCCCCCAGCCAGGCCAAGCGCGCGTG	
gp160 del V1	(1425)	GGCGCTGGGCGTGGCCCCCAGCCAGGCCAAGCGCGCGTG	
gp160 del V2	(1440)	GGCGCTGGGCGTGGCCCCCAGCCAGGCCAAGCGCGCGTG	
gp160 del V1-2	(1227)	GGCGCTGGGCGTGGCCCCCAGCCAGGCCAAGCGCGCGTG	
gp 160 del 128-194	(1272)	GGCGCTGGGCGTGGCCCCCAGCCAGGCCAAGCGCGCGTG	
gp140TM	(1521)	GGCGCTGGGCGTGGCCCCCAGCCAGGCCAAGCGCGCGTG	
gp140	(1521)	GGCGCTGGGCGTGGCCCCCAGCCAGGCCAAGCGCGCGTG	
gp140mut	(1521)	GGCGCTGGGCGTGGCCCCCAGCCAGGCCAAGCGCGCGTG	
gp120	(1521)	GGCGCTGGGCGTGGCCCCCAGCCAGGCCAAGCGCGCGTG	
Consensus	(1521)	GGCGCTGGGCGTGGCCCCCAGCCAGGCCAAGCGCGCGTG	

FIG. 6B (Sheet 8/14)

		1561	1600
gp160	(1561)	CTGCAAGCGCGAGAAAGCGCGCGCTGGGCGCTGGGCGCGCTGT	
gp160 del V1	(1465)	CTGCAAGCGCGAGAAAGCGCGCGCTGGGCGCTGGGCGCGCTGT	
gp160 del V2	(1480)	CTGCAAGCGCGAGAAAGCGCGCGCTGGGCGCTGGGCGCGCTGT	
gp160 del V1-2	(1267)	CTGCAAGCGCGAGAAAGCGCGCGCTGGGCGCTGGGCGCGCTGT	
gp 160 del 128-194	(1312)	CTGCAAGCGCGAGAAAGCGCGCGCTGGGCGCTGGGCGCGCTGT	
gp140TM	(1561)	CTGCAAGCGCGAGAAAGCGCGCGCTGGGCGCTGGGCGCGCTGT	
gp140	(1561)	CTGCAAGCGCGAGAAAGCGCGCGCTGGGCGCTGGGCGCGCTGT	
gp140mut	(1561)	CTGCAAGCGCGAGAAAGCGCGCGCTGGGCGCTGGGCGCGCTGT	
gp120	(1561)	CTGCAAGCGCGAGAAAGCGCGCGCTGGGCGCTGGGCGCGCTGT	
Consensus	(1561)	GTGCAGCGCGAGAAAGCGCGCGCTGGGCGCTGGGCGCGCTGT	
		1601	1640
gp160	(1601)	TCATCGGCTTCNCTGGGCGCGCGCGGAGCACCATGGGGCG	
gp160 del V1	(1505)	TCATCGGCTTCNCTGGGCGCGCGCGGAGCACCATGGGGCG	
gp160 del V2	(1520)	TCATCGGCTTCNCTGGGCGCGCGCGGAGCACCATGGGGCG	
gp160 del V1-2	(1307)	TCATCGGCTTCNCTGGGCGCGCGCGGAGCACCATGGGGCG	
gp 160 del 128-194	(1352)	TCATCGGCTTCNCTGGGCGCGCGCGGAGCACCATGGGGCG	
gp140TM	(1601)	TCATCGGCTTCNCTGGGCGCGCGCGGAGCACCATGGGGCG	
gp140	(1601)	TCATCGGCTTCNCTGGGCGCGCGCGGAGCACCATGGGGCG	
gp140mut	(1601)	TCATCGGCTTCNCTGGGCGCGCGCGGAGCACCATGGGGCG	
gp120	(1583)	ATATCGGCTTCNCTGGGCGCGCGCGGAGCACCATGGGGCG	
Consensus	(1601)	TCATCGGCTTCNCTGGGCGCGCGCGGAGCACCATGGGGCG	
		1641	1680
gp160	(1640)	CCGCGCTCCGTGACCGTGACCGTGACAGGCCCCGCCAGCTGCT	
gp160 del V1	(1544)	CCGCGCTCCGTGACCGTGACCGTGACAGGCCCCGCCAGCTGCT	
gp160 del V2	(1559)	CCGCGCTCCGTGACCGTGACCGTGACAGGCCCCGCCAGCTGCT	
gp160 del V1-2	(1346)	CCGCGCTCCGTGACCGTGACCGTGACAGGCCCCGCCAGCTGCT	
gp 160 del 128-194	(1391)	CCGCGCTCCGTGACCGTGACCGTGACAGGCCCCGCCAGCTGCT	
gp140TM	(1640)	CCGCGCTCCGTGACCGTGACCGTGACAGGCCCCGCCAGCTGCT	
gp140	(1640)	CCGCGCTCCGTGACCGTGACCGTGACAGGCCCCGCCAGCTGCT	
gp140mut	(1640)	CCGCGCTCCGTGACCGTGACCGTGACAGGCCCCGCCAGCTGCT	
gp120	(1600)	CCGCGCTCCGTGACCGTGACCGTGACAGGCCCCGCCAGCTGCT	
Consensus	(1641)	CCGCGCTCCGTGACCGTGACCGTGACAGGCCCCGCCAGCTGCT	
		1681	1720
gp160	(1680)	GAGCGGCATCGTGACAGCAGCAGAAACAACCTGCTGCGCGCC	
gp160 del V1	(1584)	GAGCGGCATCGTGACAGCAGCAGAAACAACCTGCTGCGCGCC	
gp160 del V2	(1599)	GAGCGGCATCGTGACAGCAGCAGAAACAACCTGCTGCGCGCC	
gp160 del V1-2	(1386)	GAGCGGCATCGTGACAGCAGCAGAAACAACCTGCTGCGCGCC	
gp 160 del 128-194	(1431)	GAGCGGCATCGTGACAGCAGCAGAAACAACCTGCTGCGCGCC	
gp140TM	(1680)	GAGCGGCATCGTGACAGCAGCAGAAACAACCTGCTGCGCGCC	
gp140	(1680)	GAGCGGCATCGTGACAGCAGCAGAAACAACCTGCTGCGCGCC	
gp140mut	(1680)	GAGCGGCATCGTGACAGCAGCAGAAACAACCTGCTGCGCGCC	
gp120	(1600)	GAGCGGCATCGTGACAGCAGCAGAAACAACCTGCTGCGCGCC	
Consensus	(1681)	GAGCGGCATCGTGACAGCAGCAGAAACAACCTGCTGCGCGCC	
		1721	1760
gp160	(1720)	ATCGAGGCCCCAGCAGCACCTGCTGACAGCTGACCGTGTGGG	
gp160 del V1	(1624)	ATCGAGGCCCCAGCAGCACCTGCTGACAGCTGACCGTGTGGG	
gp160 del V2	(1639)	ATCGAGGCCCCAGCAGCACCTGCTGACAGCTGACCGTGTGGG	
gp160 del V1-2	(1426)	ATCGAGGCCCCAGCAGCACCTGCTGACAGCTGACCGTGTGGG	
gp 160 del 128-194	(1471)	ATCGAGGCCCCAGCAGCACCTGCTGACAGCTGACCGTGTGGG	
gp140TM	(1720)	ATCGAGGCCCCAGCAGCACCTGCTGACAGCTGACCGTGTGGG	
gp140	(1720)	ATCGAGGCCCCAGCAGCACCTGCTGACAGCTGACCGTGTGGG	
gp140mut	(1720)	ATCGAGGCCCCAGCAGCACCTGCTGACAGCTGACCGTGTGGG	
gp120	(1600)	ATCGAGGCCCCAGCAGCACCTGCTGACAGCTGACCGTGTGGG	
Consensus	(1721)	ATCGAGGCCCCAGCAGCACCTGCTGACAGCTGACCGTGTGGG	

		1761	1800
gp160	(1760)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp160 del V1	(1664)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp160 del V2	(1679)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp160 del V1-2	(1466)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp 160 del 128-194	(1511)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp140TM	(1760)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp140	(1760)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp140mut	(1760)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
gp120	(1600)	-----	
Consensus	(1761)	GCATCAAGCAGCTGCAGGCCCCGCATCCTGGCCGTGGAGCG	
		1801	1840
gp160	(1800)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp160 del V1	(1704)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp160 del V2	(1719)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp160 del V1-2	(1506)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp 160 del 128-194	(1551)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp140TM	(1800)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp140	(1800)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp140mut	(1800)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
gp120	(1600)	-----	
Consensus	(1801)	CTACCTGAAGGACCAGCAGCTGCTGGGCATCTGGGGCTGC	
		1841	1880
gp160	(1840)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp160 del V1	(1744)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp160 del V2	(1759)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp160 del V1-2	(1546)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp 160 del 128-194	(1591)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp140TM	(1840)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp140	(1840)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp140mut	(1840)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
gp120	(1600)	-----	
Consensus	(1841)	AGCGGCAAGCTGATCTGCACCACCACCGTGCCCTGGAACA	
		1881	1920
gp160	(1880)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp160 del V1	(1784)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp160 del V2	(1799)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp160 del V1-2	(1586)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp 160 del 128-194	(1631)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp140TM	(1880)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp140	(1880)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp140mut	(1880)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
gp120	(1600)	-----	
Consensus	(1881)	GCAGCTGGAGCAACAAGAGCCTGACCGAGATCTGGGACAA	
		1921	1960
gp160	(1920)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp160 del V1	(1824)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp160 del V2	(1839)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp160 del V1-2	(1626)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp 160 del 128-194	(1671)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp140TM	(1920)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp140	(1920)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp140mut	(1920)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	
gp120	(1600)	-----	
Consensus	(1921)	CATGACCTGGATGGAGTGGGAGCGCGAGATCGGCAACTAC	

FIG. 4B (Sheet 10/14)

		1961	2000
gp160	(1960)	ACCGGCGCTGATCTACAAACCTGATCGAGATCGGCCAGAAAC	
gp160 del V1	(1864)	ACCGGCGCTGATCTACAAACCTGATCGAGATCGGCCAGAAAC	
gp160 del V2	(1879)	ACCGGCGCTGATCTACAAACCTGATCGAGATCGGCCAGAAAC	
gp160 del V1-2	(1666)	ACCGGCGCTGATCTACAAACCTGATCGAGATCGGCCAGAAAC	
gp 160 del 128-194	(1711)	ACCGGCGCTGATCTACAAACCTGATCGAGATCGGCCAGAAAC	
gp140TM	(1960)	ACCGGCGCTGATCTACAAACCTGATCGAGATCGGCCAGAAAC	
gp140	(1960)	ACCGGCGCTGATCTACAAACCTGATCGAGATCGGCCAGAAAC	
gp140mut	(1960)	ACCGGCGCTGATCTACAAACCTGATCGAGATCGGCCAGAAAC	
gp120	(1600)	-----	
Consensus	(1961)	ACCGGCGCTGATCTACAAACCTGATCGAGATCGGCCAGAAAC	
		2001	2040
gp160	(2000)	AGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAA	
gp160 del V1	(1904)	AGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAA	
gp160 del V2	(1919)	AGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAA	
gp160 del V1-2	(1706)	AGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAA	
gp 160 del 128-194	(1751)	AGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAA	
gp140TM	(2000)	AGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAA	
gp140	(2000)	AGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAA	
gp140mut	(2000)	AGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAA	
gp120	(1600)	-----	
Consensus	(2001)	AGCAGGAGAAGAAGCAGCAGGAGCTGCTGGAGCTGGACAA	
		2041	2080
gp160	(2040)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCACACTGG	
gp160 del V1	(1944)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCACACTGG	
gp160 del V2	(1959)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCACACTGG	
gp160 del V1-2	(1746)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCACACTGG	
gp 160 del 128-194	(1791)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCACACTGG	
gp140TM	(2040)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCACACTGG	
gp140	(2040)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCACACTGG	
gp140mut	(2040)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCACACTGG	
gp120	(1600)	-----	
Consensus	(2041)	GTGGGCCAGCCTGTGGAACCTGGTTCGACATCACCACACTGG	
		2081	2120
gp160	(2080)	CTGTGGTACATCGGCATCTTCATCATGATCGTGGGCGGGCC	
gp160 del V1	(1984)	CTGTGGTACATCGGCATCTTCATCATGATCGTGGGCGGGCC	
gp160 del V2	(1999)	CTGTGGTACATCGGCATCTTCATCATGATCGTGGGCGGGCC	
gp160 del V1-2	(1786)	CTGTGGTACATCGGCATCTTCATCATGATCGTGGGCGGGCC	
gp 160 del 128-194	(1831)	CTGTGGTACATCGGCATCTTCATCATGATCGTGGGCGGGCC	
gp140TM	(2080)	CTGTGGTACATCGGCATCTTCATCATGATCGTGGGCGGGCC	
gp140	(2080)	CTGTGGTACATCGGCATCTTCATCATGATCGTGGGCGGGCC	
gp140mut	(2080)	CTGTGGTACATCGGCATCTTCATCATGATCGTGGGCGGGCC	
gp120	(1600)	-----	
Consensus	(2081)	CTGTGGTACATCGGCATCTTCATCATGATCGTGGGCGGGCC	
		2121	2160
gp160	(2120)	TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCANNNN	
gp160 del V1	(2024)	TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCANNNN	
gp160 del V2	(2039)	TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCANNNN	
gp160 del V1-2	(1826)	TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCANNNN	
gp 160 del 128-194	(1871)	TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCANNNN	
gp140TM	(2120)	TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCANNNN	
gp140	(2092)	-----	
gp140mut	(2092)	-----	
gp120	(1600)	-----	
Consensus	(2121)	TGATCGGCCTGCGCATCGTGTTCGCCGTGCTGAGCANNNN	

FIG. 6B (Sheet 11/14)

		2161	2200
gp160	(2156)	-TCCTGAACCGCGTGGGCCAGGGCTACAGCCCCATCAGCC	
gp160 del V1	(2060)	-TCCTGAACCGCGTGGGCCAGGGCTACAGCCCCATCAGCC	
gp160 del V2	(2075)	-TCCTGAACCGCGTGGGCCAGGGCTACAGCCCCATCAGCC	
gp160 del V1-2	(1862)	-TCCTGAACCGCGTGGGCCAGGGCTACAGCCCCATCAGCC	
gp 160 del 128-194	(1907)	-TCCTGAACCGCGTGGGCCAGGGCTACAGCCCCATCAGCC	
gp140TM	(2160)	GTAAGATATTCGATCCTTTAGA-----	
gp140	(2092)	-TAAGATATTCGATCCTTTAGA-----	
gp140mut	(2092)	-TAAGATATTCGATCCTTTAGA-----	
gp120	(1600)	-----	
Consensus	(2161)	NTCGTGAACCGCGTGGGCCAGGGCTACAGCCCCATCAGCC	
		2201	2240
gp160	(2195)	TGCAGACCCGCGCTGCCCGCCCAGCGCGGCCCCGACCGCCC	
gp160 del V1	(2099)	TGCAGACCCGCGCTGCCCGCCCAGCGCGGCCCCGACCGCCC	
gp160 del V2	(2114)	TGCAGACCCGCGCTGCCCGCCCAGCGCGGCCCCGACCGCCC	
gp160 del V1-2	(1901)	TGCAGACCCGCGCTGCCCGCCCAGCGCGGCCCCGACCGCCC	
gp 160 del 128-194	(1946)	TGCAGACCCGCGCTGCCCGCCCAGCGCGGCCCCGACCGCCC	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2201)	TGCAGACCCGCGCTGCCCGCCCAGCGCGGCCCCGACCGCCC	
		2241	2280
gp160	(2235)	CGAGGGCATCGAGGAGGAGGGCGGGGAGCGCGACCGCGAC	
gp160 del V1	(2139)	CGAGGGCATCGAGGAGGAGGGCGGGGAGCGCGACCGCGAC	
gp160 del V2	(2154)	CGAGGGCATCGAGGAGGAGGGCGGGGAGCGCGACCGCGAC	
gp160 del V1-2	(1941)	CGAGGGCATCGAGGAGGAGGGCGGGGAGCGCGACCGCGAC	
gp 160 del 128-194	(1986)	CGAGGGCATCGAGGAGGAGGGCGGGGAGCGCGACCGCGAC	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2241)	CGAGGGCATCGAGGAGGAGGGCGGGGAGCGCGACCGCGAC	
		2281	2320
gp160	(2275)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
gp160 del V1	(2179)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
gp160 del V2	(2194)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
gp160 del V1-2	(1981)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
gp 160 del 128-194	(2026)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2281)	CGCAGCAACCGCCTGGTGCACGGCCTGCTGGCCCTGATCT	
		2321	2360
gp160	(2315)	GGGACGACCTGCGCAGCCTGTGCCTGTTTACGCTACCAACCG	
gp160 del V1	(2219)	GGGACGACCTGCGCAGCCTGTGCCTGTTTACGCTACCAACCG	
gp160 del V2	(2234)	GGGACGACCTGCGCAGCCTGTGCCTGTTTACGCTACCAACCG	
gp160 del V1-2	(2021)	GGGACGACCTGCGCAGCCTGTGCCTGTTTACGCTACCAACCG	
gp 160 del 128-194	(2066)	GGGACGACCTGCGCAGCCTGTGCCTGTTTACGCTACCAACCG	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2321)	GGGACGACCTGCGCAGCCTGTGCCTGTTTACGCTACCAACCG	

FIG. 6B (Sheet 12/14)

		2361	2400
gp160	(2355)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
gp160 del V1	(2259)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
gp160 del V2	(2274)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
gp160 del V1-2	(2061)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
gp 160 del 128-194	(2106)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2361)	CCTGCGCGACCTGCTGCTGATCGTGGCCCGCATCGTGGAG	
		2401	2440
gp160	(2395)	CTGCTGGGCGCCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
gp160 del V1	(2299)	CTGCTGGGCGCCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
gp160 del V2	(2314)	CTGCTGGGCGCCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
gp160 del V1-2	(2101)	CTGCTGGGCGCCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
gp 160 del 128-194	(2146)	CTGCTGGGCGCCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2401)	CTGCTGGGCGCCCGCGGCTGGGAGGCCCTGAAGTACTGGT	
		2441	2480
gp160	(2435)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
gp160 del V1	(2339)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
gp160 del V2	(2354)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
gp160 del V1-2	(2141)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
gp 160 del 128-194	(2186)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2441)	GGAACCTGCTGCAGTACTGGAGCCAGGAGCTGAAGAGCAG	
		2481	2520
gp160	(2475)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
gp160 del V1	(2379)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
gp160 del V2	(2394)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
gp160 del V1-2	(2181)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
gp 160 del 128-194	(2226)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2481)	CGCCGTGAGCCTGTTCAACGCCACCGCCATCGCCGTGGCC	
		2521	2560
gp160	(2515)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	
gp160 del V1	(2419)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	
gp160 del V2	(2434)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	
gp160 del V1-2	(2221)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	
gp 160 del 128-194	(2266)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2521)	GAGGGCACCGACCGCATCATCGAGATCGTGCAGCGCATCT	

FIG.66B (Sheet 13/14)

		2561	2600
gp160	(2555)	TCCGCGCCGTGATCCACATCCCCCGCCGCATCCGCCAGGG	
gp160 del V1	(2459)	TCCGCGCCGTGATCCACATCCCCCGCCGCATCCGCCAGGG	
gp160 del V2	(2474)	TCCGCGCCGTGATCCACATCCCCCGCCGCATCCGCCAGGG	
gp160 del V1-2	(2261)	TCCGCGCCGTGATCCACATCCCCCGCCGCATCCGCCAGGG	
gp 160 del 128-194	(2306)	TCCGCGCCGTGATCCACATCCCCCGCCGCATCCGCCAGGG	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2561)	TCCGCGCCGTGATCCACATCCCCCGCCGCATCCGCCAGGG	
		2601	2640
gp160	(2595)	CCTGGAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA	
gp160 del V1	(2499)	CCTGGAGCGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA	
gp160 del V2	(2514)	CCTGGAGCGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA	
gp160 del V1-2	(2301)	CCTGGAGCGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA	
gp 160 del 128-194	(2346)	CCTGGAGCGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2601)	CCTGGAGCGCGCCCTGCTGTAAGATATCGGATCCTCTAGA	
		2641	2680
gp160	(2635)	AAGCCATGGATATCGGATCCACTACGCGTTAGAGCTCGCT	
gp160 del V1	(2539)	-----	
gp160 del V2	(2554)	-----	
gp160 del V1-2	(2341)	-----	
gp 160 del 128-194	(2386)	-----	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2641)	NN	
		2681	
gp160	(2675)	GATCAGCT	
gp160 del V1	(2539)	-----	
gp160 del V2	(2554)	-----	
gp160 del V1-2	(2341)	-----	
gp 160 del 128-194	(2386)	-----	
gp140TM	(2182)	-----	
gp140	(2113)	-----	
gp140mut	(2113)	-----	
gp120	(1600)	-----	
Consensus	(2681)	NNNNNNNN	

FIG. 6B (Sheet 14/14)

Time (hours)	Group 1 Concentration (mg/100 ml)	Group 2 Concentration (mg/100 ml)
0	0	0
4	~500	~1000
8	~1200	~800
12	~2200	~1500
16	~2200	~2200
28	~1800	~16000
30	~50000	~10000
32	~48000	~8000

FIG.67

HIV-1SF2 wt RT (PISPIET-->GIRKVL)

CCCATTAGTCCTATTGAAACTGTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCCAAA
GTTAAGCAATGGCCATTGACAGAAGAAAAATAAAAGCATTAGTAGAGATATGTACAGAA
ATGGAAAAGGAAGGGAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAGTA
TTTGCTATAAAGAAAAAGACAGTACTAAATGGAGAAAAC TAGTAGATTTTCAGAGAACTT
AATAAAAGAACTCAAGACTTCTGGGAAGTTT CAGTTAGGAATACCACACCCCGCAGGGTTA
AAAAAGAAAAAATCAGTAACAGTATTGGATGTGGGTGATGCATACTTTTCAGTTCCCTTA
GATAAAGACTTTAGAAAGTATACTGCATTTACCATACCTAGTATAAACAATGAGACACCA
GGGATTAGATATCAGTACAATGTGCTGCCACAGGGATGGAAAGGATCACCAGCAATATT
CAAAGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAAAACAGAATCCAGACATAGTTATC
TATCAAtacatggatgatTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACA
AAAATAGAGGAACTGAGACAGCATCTGTTGAGGTGGGGATTTACCACACCAGACAAAAAA
CATCAGAAAGAACCTCCATTCTTTggatgggttatGAACTCCATCCTGATAAATGGACA
GTACAGCCTATAATGCTGCCAGAAAAAGACAGCTGGACTGTCAATGACATACAGAAGTTA
GTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGATTAAAGTAAAGCAGTTATGT
AAACTCCTTAGAGGAACCAAAGCACTAACAGAAGTAATACCACTAACAGAAGAAGCAGAG
CTAGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCAGTACATGAAGTATATTATGAC
CCATCAAAAGACTTAGTAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATCAA
ATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAAAGTATGCAAGGATGAGGGGTGCC
CACACTAATGATGTAAAACAGTTAACAGAGGCAGTGCAAAAAGTATCCACAGAAAGCATA
GTAATATGGGGAAAGATTCTTAAATTTAAACTACCCATACAAAAGGAAACATGGGAAGCA
TGGTGGATGGAGTATTGGCAAGCTACCTGGATTCTCTGAGTGGGAGTTTGTCAATACCCCT
CCCTTAGTGAAATTATGGTACCAGTTAGAGAAAGAACCCATAGTAGGAGCAGAACTTTC
TATGTAGATGGGGCAGCTAATAGGGAGACTAAATTAGGAAAAGCAGGATATGTTACTGAC
AGAGGAAGACAAAAAGTTGTCTCCATAGCTGACACAACAAATCAGAAGACTGAATTACAA
GCAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAACATAGTAACAGACTCACAA
TATGCATTAGGAATCATTCAAGCACACCAGATAAGAGTGAATCAGAGTTAGTCAGTCAA
ATAATAGAGCAGTTAATAAAAAAGGAAAAGGTCTACCTGGCATGGGTACCAGCACACAAA
GGAATTGGAGGAAATGAACAAGTAGATAAATTAGTCAGTGCTGGAATCAGGAAAGTACTA

FIG. 68 (SEQ ID NO:77)

GagProtMod.SF2 (GP1)

GTCGACGCCACCATGGGCGCCCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGTGG
GAGAAGATCCGCCTGCGCCCCGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGG
GCCAGCCGCGAGCTGGAGCGCTTCGCCGTGAACCCCGGCCTGCTGGAGACCAGCGAGGGC
TGCCGCCAGATCCTGGGCCAGCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGC
AGCCTGTACAACACCGTGGCCACCCTGTACTGCGTGCACCAGCGCATCGACGTCAAGGAC
ACCAAGGAGGGCCCTGGAGAAGATCGAGGAGGAGCAGAACAAGTCCAAGAAGAAGGCCAG
CAGGCCGCGCCGCGCCGCGGCACCGGCAACAGCAGCCAGGTGAGCCAGAACTACCCCATC
GTGCAGAACCTGCAGGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCCCTGAACGCC
TGGGTGAAGGTGGTGGAGGAGAAGGCCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCC
CTGAGCGAGGGCGCCACCCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCAC
CAGGCCGCCATGCAGATGCTGAAGGAGACCATCAACGAGGAGGCCGCGGAGTGGGACCGC
GTGCACCCCGTGCACGCCGCCCCATCGCCCCCGGCCAGATGCGCGAGCCCCGCGGCAGC
GACATCGCCGGCACCACCAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCAACAACCCC
CCCATCCCCGTGGGCGAGATCTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCGTG
CGGATGTACAGCCCCACCAGCATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCCGC
GACTACGTGGACCGCTTCTACAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGAAG
AACTGGATGACCGAGACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTG
AAGGCTCTCGGCCCCGCGGCCACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGC
GGCCCCGGCCACAAGGCCCGCGTGCTGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCG
ACCATCATGATGCAGCGCGGCAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAAC
TGCGGCAAGGAGGGCCACACCGCCAGGAAGTGCCGCGCCCCCGCAAGAAGGGCTGCTGG
CGCTGCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTA
GGGAAGATCTGGCCTTCTTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAG
CCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAGGAGAAAACAACTCCCTCTCAG
AAGCAGGAGCCGATAGACAAGGAAGTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGC
AACGACCCCTCGTCACAGTAAGGATCGGCGGCCAGCTCAAGGAGGCGCTGCTCGACACCG
GCGCCGACGACACCGTGCTGGAGGAGATGAACCTGCCCGGCAAGTGGAAGCCCAAGATGA
TCGGCGGGATCGGGGGCTTCATCAAGGTGCGGCAGTACGACCAGATCCCCGTGGAGATCT
GCGGCCACAAGGCCATCGGCACCGTGCTGGTGGGCCCCACCCCGTGAACATCATCGGCC
GCAACCTGCTGACCCAGATCGGCTGCACCCTGAACTTCCCCATCAGCCCCATCGAGACGG
TGCCCGTGAAGCTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCCCTGTAAG
AATTC

FIG. 69 (SEQ ID NO:78)

GagProtMod.SF2 (GP2)

GTCGACGCCACCATGGGCGCCCGCGCCAGCGTGCTGAGCGGCGGCGAGCTGGACAAGTGG
 GAGAAGATCCGCCTGCGCCCCGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGG
 GCCAGCCGCGAGCTGGAGCGCTTCGCCGTGAACCCCGGCCTGCTGGAGACCAGCGAGGGC
 TGCCGCCAGATCCTGGGCCAGCTGCAGCCCAGCCTGCAGACCGGCAGCGAGGAGCTGCGC
 AGCCTGTACAACACCGTGGCCACCCTGTACTGCGTGCACCAGCGCATCGACGTCAAGGAC
 ACCAAGGAGGGCCCTGGAGAAGATCGAGGAGGAGCAGAACAAGTCCAAGAAGAAGGCCAG
 CAGGCCGCCGCCGCCGCCGGCACC GGCAACAGCAGCCAGGTGAGCCAGAACTACCCCATC
 GTGCAGAACCTGCAGGGCCAGATGGTGCACCAGGCCATCAGCCCCCGCACCCCTGAACGCC
 TGGGTGAAGGTGGTGGAGGAGAAGGCCCTTCAGCCCCGAGGTGATCCCCATGTTTCAGCGCC
 CTGAGCGAGGGCGCCACCCCCCAGGACCTGAACACGATGTTGAACACCGTGGGCGGCCAC
 CAGGCCGCCATGCAGATGCTGAAGGAGACCATCAACGAGGAGGCCGCCGAGTGGGACCGC
 GTGCACCCCGTGCACGCCGGCCCCATCGCCCCGGCCAGATGCGCGAGCCCCCGCGGCAGC
 GACATCGCCGGCACCAACAGCACCCCTGCAGGAGCAGATCGGCTGGATGACCAACAACCCC
 CCCATCCCCGTGGGCGAGATCTACAAGCGGTGGATCATCCTGGGCCTGAACAAGATCGTG
 CGGATGTACAGCCCCACCAGCATCCTGGACATCCGCCAGGGCCCCAAGGAGCCCTTCCGC
 GACTACGTGGACCGCTTCTACAAGACCCTGCGCGCTGAGCAGGCCAGCCAGGACGTGAAG
 AACTGGATGACCGAGACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTG
 AAGGCTCTCGGCCCCGCGGCCACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGC
 GGCCCCGGCCACAAGGCCCGCGTGCTGGCCGAGGCGATGAGCCAGGTGACGAACCCGGCG
 ACCATCATGATGCAGCGCGGCAACTTCCGCAACCAGCGGAAGACCGTCAAGTGCTTCAAC
 TGC GGCAAGGAGGGCCACACCGCCAGGAAGTCCCGCGCCCCCGCAAGAAGGGCTGCTGG
 CGCTGCGGCCGCGAAGGACACCAAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTA
 GGAAGATCTGGCCTTCTCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAG
 CCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAGGAGAAAACAACCTCCCTCTCAG
 AAGCAGGAGCCGATAGACAAGGAAGTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGC
 AACGACCCCTCGTCACAGTAAGGATCGGGGGGCAACTCAAGGAAGCGCTGCTCGATACAG
 GAGCAGATGATACAGTATTAGAAGAAATGAATTTGCCAGGAAAATGGAAACCAAAAATGA
 TAGGGGGGATCGGGGGCTTCATCAAGGTGAGGCAGTACGACCAGATACCTGTAGAAATCT
 GTGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAA
 GAAATCTGTTGACCCAGATCGGCTGCACCTTGAAGTTCCCATCAGCCCTATTGAGACGG
 TGCCCGTGAAGTTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAATGGCCATTGTAAG
 AATTC

FIG. 70 (SEQ ID NO:79)

FS(+)_ProtInact_RTpt_YM

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTAGGGA
AGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCAA
CAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAGGAGAAAACAACCTCCCTCTCAGAAGC
AGGAGCCGATAGACAAGGAACTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGCAACG
ACCCCTCGTCACAATAAGGATCGGGGGGCAACTCAAGGAAGCGCTGCTCGATACAGGAGC
AGATGATACAGTATTAGAAGAAATGAATTTGCCAGGAAAATGGAAACCAAAAATGATAGG
GGGGATCGGGGGCTTCATCAAGGTGAGGCAGTACGACCAGATACCTGTAGAAATCTGTGG
ACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAA
TCTGTTGACCCAGATCGGCTGCACCTTGAACCTTCCCCATCAGCCCTATTGAGACGGTGCC
CGTGAAGTTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAATGGCCATTGACCGAGGA
GAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCAA
GATCGGCCCCGAGAACCCCTACAACACCCCGTGTTGCCATCAAGAAGAAGGACAGCAC
CAAGTGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGA
GGTGCAGCTGGGCATCCCCACCCCGCGGCTGAAGAAGAAGAAGAGCGTGACCGTGCT
GGACGTGGGCGACGCCTACTTCAGCGTGCCCTGGACAAGGACTTCCGCAAGTACACCGC
CTTCACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAGTACAACGTGCT
GCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGGA
GCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGGCCCCCCTGTACGTGGGCAG
CGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACCTGCTGCG
CTGGGGCTTCACCACCCCGACAAGAAGCACCAGAAGGAGCCCCCTTCCTGTGGATGGG
CTACGAGCTGCACCCGACAAGTGGACCGTGCAGCCCATCATGCTGCCCCGAGAAGGACAG
CTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCTA
CGCCGGCATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCGA
GGTGATCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGAA
GGAGCCCGTGACGAGGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGAA
GCAGGGCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGAC
CGGCAAGTACGCCCCGATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGACCGAGGC
CGTGCGAGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGTTCAAGCT

FIG. 71 (SEQ ID NO:80)

GCCCATCCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCACCTGGAT
CCCCGAGTGGGAGTTCGTGAACACCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAA
GGAGCCCATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACCGCGAGACCAA
GCTGGGCAAGGCCGGCTACGTGACCGACCGGGGCCGGCAGAAGGTGGTGAGCATCGCCGA
CACCACCAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACAGCGGCCT
GGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCCAGCCGA
CAAGAGCGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGT
GTACCTGGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGGACAAGCT
GGTGAGCGCCGGCATCCGCAAGGTGCTGTTCTTGAACGGCATCGATGGCGGCATCGTGAT
CTACCAGTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATTAAAAGCT
TCCCGGGGCTAGCACCGGTGAATTC

FIG. 71 (CONT'D.) (SEQ ID NO:80)

FS(+)_ProtInact_RTtop_YMWM

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTTTTAGGGA
 AGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCAA
 CAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGGAGGAGAAAACAACTCCCTCTCAGAAGC
 AGGAGCCGATAGACAAGGAACTGTATCCTTTAACTTCCCTCAGATCACTCTTTGGCAACG
 ACCCTCTCGTCACAATAAGGATCGGGGGGCAACTCAAGGAAGCGCTGCTCGATACAGGAGC
 AGATGATACAGTATTAGAAGAAATGAATTTGCCAGGAAAATGGAAACCAAAAATGATAGG
 GGGGATCGGGGGCTTCATCAAGGTGAGGCAGTACGACCAGATACCTGTAGAAATCTGTGG
 ACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAAA
 TCTGTTGACCCAGATCGGCTGCACCTTGAAGTTCCCCATCAGCCCTATTGAGACGGTGCC
 CGTGAAGTTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAATGGCCATTGACCGAGGA
 GAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCAA
 GATCGGCCCCGAGAACCCCTACAACACCCCCGTGTTCCGCCATCAAGAAGAAGGACAGCAC
 CAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGGA
 GGTGCAGCTGGGCATCCCCACCCCGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGCT
 GGACGTGGGCGACGCCTACTTCAGCGTGCCCCTGGACAAGGACTTCCGCAAGTACACCGC
 CTTACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAGTACAACGTGCT
 GCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGGA
 GCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGGCCCCCTGTACGTGGGCAG
 CGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACCTGCTGCG
 CTGGGGCTTCACCACCCCCGACAAGAAGCACCAGAAGGAGCCCCCTTCCTGCCCATCGA
 GCTGCACCCCGACAAGTGGACCGTGCAGCCCATCATGCTGCCCCGAGAAGGACAGCTGGAC
 CGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCTACGCCGG
 CATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCGAGGTGAT
 CCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGAAGGAGCC
 CGTGACAGAGGTGTACTACGACCCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGGG
 CCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCAA
 GTACGCCCCGATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGCA
 GAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGTTCAAGCTGCCCAT

FIG. 72 (SEQ ID NO:81)

CCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCACCTGGATCCCCGA
GTGGGAGTTTCGTGAACACCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCC
CATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACCGCGAGACCAAGCTGGG
CAAGGCCGGCTACGTGACCGACCGGGGCGGCAGAAAGGTGGTGAGCATCGCCGACACCAC
CAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACAGCGGCCTGGAGGT
GAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCCAGCCCGACAAGAG
CGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACCT
GGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGGACAAGCTGGTGAG
CGCCGGCATCCGCAAGGTGCTGTTCCCTGAACGGCATCGATGGCGGCATCGTGATCTACCA
GTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATTAAAAGCTTCCCGG
GGCTAGCACCGGTGAATTC

FIG. 72 (CONT'D.) (SEQ ID NO:81)

FS(-)_ProtMod_RTopt_YM

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTCTTCCGCG
AGGACCTGGCCTTCTTGCAGGGCAAGGCCGCGAGTTCAGCAGCGAGCAGACCCGCGCCA
ACAGCCCCACCCGCCGCGAGCTGCAGGTGTGGGGCGGCGAGAACAACAGCCTGAGCGAGG
CCGGCGCCGACCGCCAGGGCACCGTGAGCTTCAACTTCCCCCAGATCACCTGTGGCAGC
GCCCCCTGGTGACCATCAGGATCGGCGGCCAGCTCAAGGAGGCGCTGCTCGACACCGGCG
CCGACGACACCGTGCTGGAGGAGATGAACCTGCCCGGCAAGTGGAAGCCCAAGATGATCG
GCGGGATCGGGGGCTTCATCAAGGTGCGGCAGTACGACCAGATCCCCGTGGAGATCTGCG
GCCACAAGGCCATCGGCACCGTGCTGGTGGGCCCCACCCCCGTGAACATCATCGGCCGCA
ACCTGCTGACCCAGATCGGCTGCACCCTGAACTTCCCCATCAGCCCCATCGAGACGGTGC
CCGTGAAGCTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCCCCTGACCGAGG
AGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCA
AGATCGGGCCCCGAGAACCCCTACAACACCCCCGTGTTCCGCATCAAGAAGAAGGACAGCA
CCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGG
AGGTGCAGCTGGGCATCCCCACCCCGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGC
TGGACGTGGGCGACGCCTACTTCAGCGTGCCCCCTGGACAAGGACTTCCGCAAGTACACCG
CCTTCACCATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAGTACAACGTGC
TGCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGG
AGCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGGCCCCCCTGTACGTGGGCA
GCGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACCTGCTGC
GCTGGGGCTTCACCACCCCCGACAAGAAGCACCAGAAGGAGCCCCCCTTCTGTGGATGG
GCTACGAGCTGCACCCCGACAAGTGGACCGTGCGAGCCCATCATGCTGCCCCGAGAAGGACA
GCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCT
ACGCCGGCATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCG
AGGTGATCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGA
AGGAGCCCGTGACGAGGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGATCCAGA
AGCAGGGCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGA
CCGGCAAGTACGCCCCGATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGACCGAGG
CCGTGCAGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGTTCAAGC

FIG. 73 (SEQ ID NO:82)

FS(-)_ProtMod_RTopt_YMWM

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTCTTCCGCG
AGGACCTGGCCTTCCTGCAGGGCAAGGCCCGCGAGTTCAGCAGCGAGCAGACCCGCGCCA
ACAGCCCCACCCGCCGCGAGCTGCAGGTGTGGGGCGGCGAGAACAACAGCCTGAGCGAGG
CCGGCGCCGACCGCCAGGGCACCGTGAGCTTCAACTTCCCCCAGATCACCTGTGGCAGC
GCCCCCTGGTGACCATCAGGATCGGCGGCCAGCTCAAGGAGGCGCTGCTCGACACCGGCG
CCGACGACACCGTGCTGGAGGAGATGAACCTGCCCCGCAAGTGGAAGCCCAAGATGATCG
GCGGGATCGGGGGCTTCATCAAGGTGCGGCAGTACGACCAGATCCCCGTGGAGATCTGCG
GCCACAAGGCCATCGGCACCGTGCTGGTGGGCCCCACCCCCGTGAACATCATCGGCCGCA
ACCTGCTGACCCAGATCGGCTGCACCCTGAACTTCCCCATCAGCCCCATCGAGACGGTG
CCGTGAAGCTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCCCCTGACCGAGG
AGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCA
AGATCGGCCCCGAGAACCCTACAACACCCCCGTGTTCCGCATCAAGAAGAAGGACAGCA
CCAAGTGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGG
AGGTGCAGCTGGGCATCCCCCACCCCGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTG
TGGACGTGGGCGACGCCTACTTCAGCGTGCCCCCTGGACAAGGACTTCCGCAAGTACACCG
CCTTCACCATCCCCAGCATCAACAACGAGACCCCCGGCATCCGCTACCAGTACAACGTGC
TGCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGG
AGCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGGCCCCCTGTACGTGGGCA
GCGACCTGGAGATCGGCCAGCACCCGCACCAAGATCGAGGAGCTGCGCCAGCACCTGCTGC
GCTGGGGCTTCACCACCCCCGACAAGAAGCACCAGAAGGAGCCCCCTTCTGCCCATCG
AGCTGCACCCCGACAAGTGGACCGTGACGCCATCATGCTGCCCCGAGAAGGACAGCTGGA
CCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCTACGCCG
GCATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCCTGACCGAGGTGA
TCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGATCCTGAAGGAGC
CCGTGCACGAGGTGTACTACGACCCCAGCAAGGACCTGGTGGCCGAGATCCAGAAGCAGG
GCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACCTGAAGACCGGCA
AGTACGCCCCGATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGACCGAGGCCGTGC
AGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGTTCAAGCTGCCCA

FIG. 74 (SEQ ID NO:83)

TCCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCACCTGGATCCCCG
 AGTGGGAGTTTCGTGAACACCCCCCCCCCTGGTGAAGCTGTGGTACCAGCTGGAGAAGGAGC
 CCATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACC GCGAGACCAAGCTGG
 GCAAGGCCGGCTACGTGACCGACCGGGGGCCGGCAGAAGGTGGTGAGCATCGCCGACACCA
 CCAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACAGCGGCCTGGAGG
 TGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCCAGCCCGACAAGA
 GCGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGGAGAAGGTGTACC
 TGGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGGACAAGCTGGTGA
 GCGCCGGCATCCGCAAGGTGCTGTTCTGAACGGCATCGATGGCGGCATCGTGATCTACC
 AGTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATTAAAAGCTTCCCG
 GGGCTAGCACCGGTGAATTC

FIG. 74 (CONT'D.) (SEQ ID NO:83)

FS(-)_ProtMod_RTopt(+)

GCGGCCGCGAAGGACACCAAATGAAAGATTGCACTGAGAGACAGGCTAATTTCTTCCGCG
 AGGACCTGGCCTTCCTGCAGGGCAAGGCCCCGCGAGTTCAGCAGCGAGCAGACCCGCGCCA
 ACAGCCCCACCCGCCGCGAGCTGCAGGTGTGGGGCGGCGAGAACAACAGCCTGAGCGAGG
 CCGGCGCCGACCGCCAGGGCACCGTGAGCTTCAACTTCCCCCAGATCACCTGTGGCAGC
 GCCCCCTGGTGACCATCAGGATCGGCGGCCAGCTCAAGGAGGCGCTGCTCGACACCGGCG
 CCGACGACACCGTGCTGGAGGAGATGAACCTGCCCGCAAGTGGAAGCCCAAGATGATCG
 GCGGGATCGGGGGCTTCATCAAGGTGCGGCAGTACGACCAGATCCCCGTGGAGATCTGCG
 GCCACAAGGCCATCGGCACCGTGCTGGTGGGCCCCACCCCGTGAACATCATCGGCCGCA
 ACCTGCTGACCCAGATCGGCTGCACCCTGAACTTCCCCATCAGCCCCATCGAGACGGTG
 CCGTGAAGCTGAAGCCGGGGATGGACGGCCCCAAGGTCAAGCAGTGGCCCCTGACCGAGG
 AGAAGATCAAGGCCCTGGTGGAGATCTGCACCGAGATGGAGAAGGAGGGCAAGATCAGCA
 AGATCGGCCCCGAGAACCCCTACAACACCCCGTGTTCCGCATCAAGAAGAAGGACAGCA
 CCAAGTGGCGCAAGCTGGTGGACTTCCGCGAGCTGAACAAGCGCACCCAGGACTTCTGGG
 AGGTGCAGCTGGGCATCCCCACCCCGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGC
 TGGACGTGGGCGACGCCTACTTCAGCGTGCCCTGGACAAGGACTTCCGCAAGTACACCG
 CCTTCACCATCCCCAGCATCAACAACGAGACCCCGGCATCCGCTACCAGTACAACGTGC
 TGCCCCAGGGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACCAAGATCCTGG
 AGCCCTTCCGCAAGCAGAACCCCGACATCGTGATCTACCAGTACATGGACGACCTGTACG
 TGGGCAGCGACCTGGAGATCGGCCAGCACCGCACCAAGATCGAGGAGCTGCGCCAGCACC
 TGCTGCGCTGGGGCTTCACCACCCCGACAAGAAGCACCAGAAGGAGCCCCCTTCTGT
 GGATGGGCTACGAGCTGCACCCCGACAAGTGGACCGTGAGCCCATCATGCTGCCCCGAGA
 AGGACAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCC
 AGATCTACGCCGGCATCAAGGTGAAGCAGCTGTGCAAGCTGCTGCGCGGCACCAAGGCCC
 TGACCGAGGTGATCCCCCTGACCGAGGAGGCCGAGCTGGAGCTGGCCGAGAACCGCGAGA
 TCCTGAAGGAGCCCGTGACAGAGGTGTACTACGACCCAGCAAGGACCTGGTGGCCGAGA
 TCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAGATCTACCAGGAGCCCTTCAAGAACC
 TGAAGACCGGCAAGTACGCCCCGATGCGCGGCGCCACACCAACGACGTGAAGCAGCTGA
 CCGAGGCCGTGAGAAGGTGAGCACCGAGAGCATCGTGATCTGGGGCAAGATCCCCAAGT
 TCAAGCTGCCCATCCAGAAGGAGACCTGGGAGGCCTGGTGGATGGAGTACTGGCAGGCCA
 CCTGGATCCCCGAGTGGGAGTTCGTGAACACCCCCCCCCTGGTGAAGCTGTGGTACCAGC
 TGGAGAAGGAGCCCATCGTGGGCGCCGAGACCTTCTACGTGGACGGCGCCGCCAACC

FIG. 75 (SEQ ID NO:84)

AGACCAAGCTGGGCAAGGCCGGCTACGTGACCGACCGGGGCCGCAGAAGGTGGTGAGCA
TCGCCGACACCACCAACCAGAAGACCGAGCTGCAGGCCATCCACCTGGCCCTGCAGGACA
GCGGCCTGGAGGTGAACATCGTGACCGACAGCCAGTACGCCCTGGGCATCATCCAGGCCC
AGCCCGACAAGAGCGAGAGCGAGCTGGTGAGCCAGATCATCGAGCAGCTGATCAAGAAGG
AGAAGGTGTACCTGGCCTGGGTGCCCCGCCACAAGGGCATCGGCGGCAACGAGCAGGTGG
ACAAGCTGGTGAGCGCCGGCATCCGCAAGGTGCTGTTCCCTGAACGGCATCGATGGCGGCA
TCGTGATCTACCAGTACATGGACGACCTGTACGTGGGCAGCGGCGGCCCTAGGATCGATT
AAAAGCTTCCCGGGGCTAGCACCGGTGAATTC

FIG. 75 (CONT'D.) (SEQ ID NO:84)

FIG. 75 (CONT'D.) (SEQ ID NO:84)

Tat_wt_SF162 (wildtype)

ATGGAGCCAGTAGATCCTAGATTAGAGCCCTGGAAGCATCCAGGAAGTCAGCCTAAGA
CTGCTTGTACAAATTGCTATTGTAAAAAGTGTTGCTTTCATTGCCAAGTTTGTTTCATAAC
AAAAGGCTTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCCT
CCAGACAGTGAGGTTTCATCAAGTTTCTCTACCAAAGCAACCCGCTTCCCAGCCCCAAGG
GGACCCGACAGGCCCGAAGGAATCGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGA
TCCAGTCCATTAG

FIG. 76 (SEQ ID NO:85)

650221-21-23-460

Tat_SF162

MEPVDPRLEPWKHPGSQPKTACTNCYCKKCCFHCQVCFITKGLGISYGRKKRRQRRRAPPDSE
VHQVSLPKQPASQPQGDPTGPKESKKKVERETETDPVH

FIG. 77 (SEQ ID NO:86)

Seq. ID: 86

[illegible]

FIG. 78 (SEQ ID NO:87)

Tat_Cys22_SF162_opt

ATGGAGCCCGTGGACCCCCGCCTGGAGCCCTGGAAGCACCCCGGCAGCCAGCCCAAGAC
CGCCgGCACCAACTGCTACTGCAAGAAGTGCTGCTTCCACTGCCAGGTGTGCTTCATCACCA
AGGGCCTGGGCATCAGCTACGGCCGCAAGAAGCGCCGCCAGCGCCGCCGCGCCCCCCCC
GACAGCGAGGTGCACCAGGTGAGCCTGCCCAAGCAGCCGCCAGCCAGCCCCAGGGCGA
CCCCACCGGCCCCAAGGAGAGCAAGAAGAAGGTGGAGCGCGAGACCGAGACCGACCCCG
TGCACTAG

FIG. 79 (SEQ ID NO:88)

66022723460

FIG. 80A

660E2T "3T554450

Alignment GagMod vs GP1_GP2

	Section 6									
	381	390	400	410	420	430	440	450		
GagMod.SF2	(381)									
GagProtMod.SF2(GP1)	(381)									
GagProtMod.SF2(GP2)	(381)									
Consensus	(381)									
	Section 7									
	457	470	480	490	500	510	520	532		
GagMod.SF2	(457)									
GagProtMod.SF2(GP1)	(457)									
GagProtMod.SF2(GP2)	(457)									
Consensus	(457)									
	Section 8									
	533	540	550	560	570	580	590	608		
GagMod.SF2	(533)									
GagProtMod.SF2(GP1)	(533)									
GagProtMod.SF2(GP2)	(533)									
Consensus	(533)									
	Section 9									
	609	620	630	640	650	660	670	684		
GagMod.SF2	(609)									
GagProtMod.SF2(GP1)	(609)									
GagProtMod.SF2(GP2)	(609)									
Consensus	(609)									
	Section 10									
	685	690	700	710	720	730	740	750	760	
GagMod.SF2	(685)									
GagProtMod.SF2(GP1)	(685)									
GagProtMod.SF2(GP2)	(685)									
Consensus	(685)									

FIG. 80B

660E2T " 5T33460

Alignment GagMod vs GP1_GP2

	770	780	790	800	810	820	836	Section 11
GagMod.SF2 (761)	761							
GagMod.SF2(GP1) (761)	761	ACAAACCC	CCCAATCC	CCCGTGGG	CGGAGATCT	ACAAAGCGGT	GGGCTGAACA	AGATCGT
GagMod.SF2(GP2) (761)	761	ACAAACCC	CCCAATCC	CCCGTGGG	CGGAGATCT	ACAAAGCGGT	GGGCTGAACA	AGATCGT
Consensus (761)	761	ACAAACCC	CCCAATCC	CCCGTGGG	CGGAGATCT	ACAAAGCGGT	GGGCTGAACA	AGATCGT
								Section 12
GagMod.SF2 (837)	837							
GagMod.SF2(GP1) (837)	837	CAGCCCC	ACCAAGCATC	CTGGACAT	CCCGCCAGG	CCCCCAAGG	AGCCCCCT	TCCCGGACT
GagMod.SF2(GP2) (837)	837	CAGCCCC	ACCAAGCATC	CTGGACAT	CCCGCCAGG	CCCCCAAGG	AGCCCCCT	TCCCGGACT
Consensus (837)	837	CAGCCCC	ACCAAGCATC	CTGGACAT	CCCGCCAGG	CCCCCAAGG	AGCCCCCT	TCCCGGACT
								Section 13
GagMod.SF2 (913)	913							
GagMod.SF2(GP1) (913)	913	ACCC	TGCGCGCT	GAGCAGG	CCAGCCAGG	ACGTGGA	AACTGGAT	GACCCGAG
GagMod.SF2(GP2) (913)	913	ACCC	TGCGCGCT	GAGCAGG	CCAGCCAGG	ACGTGGA	AACTGGAT	GACCCGAG
Consensus (913)	913	ACCC	TGCGCGCT	GAGCAGG	CCAGCCAGG	ACGTGGA	AACTGGAT	GACCCGAG
								Section 14
GagMod.SF2 (989)	989							
GagMod.SF2(GP1) (989)	989	CCGACTG	CAAGACCA	ATCC	TGAAGGCT	CTCGGCC	CCCGCGGCC	ACCC
GagMod.SF2(GP2) (989)	989	CCGACTG	CAAGACCA	ATCC	TGAAGGCT	CTCGGCC	CCCGCGGCC	ACCC
Consensus (989)	989	CCGACTG	CAAGACCA	ATCC	TGAAGGCT	CTCGGCC	CCCGCGGCC	ACCC
								Section 15
GagMod.SF2 (1065)	1065							
GagMod.SF2(GP1) (1065)	1065	GGCGGG	CCCCCGG	CCACAA	AGGCCCG	CGTGTG	CGCCGAG	CGGATG
GagMod.SF2(GP2) (1065)	1065	GGCGGG	CCCCCGG	CCACAA	AGGCCCG	CGTGTG	CGCCGAG	CGGATG
Consensus (1065)	1065	GGCGGG	CCCCCGG	CCACAA	AGGCCCG	CGTGTG	CGCCGAG	CGGATG

FIG. 80C

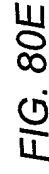
650E2T "3155460

Alignment GagMod vs GP1_GP2

	Section 16										
	1150	1160	1170	1180	1190	1200					1216
(1141) 1141											
GagMod.SF2(1141)	CAGCGCGGCAACTTCCGCAACCAAGCGAAGACCGTCAAGTCTTCAACTGCGGCAAGGAGGCGCCACACCCGAGGA										
GagProfMod.SF2(GP1)(1141)	CAGCGCGGCAACTTCCGCAACCAAGCGAAGACCGTCAAGTCTTCAACTGCGGCAAGGAGGCGCCACACCCGAGGA										
GagProfMod.SF2(GP2)(1141)	CAGCGCGGCAACTTCCGCAACCAAGCGAAGACCGTCAAGTCTTCAACTGCGGCAAGGAGGCGCCACACCCGAGGA										
Consensus(1141)	CAGCGCGGCAACTTCCGCAACCAAGCGAAGACCGTCAAGTCTTCAACTGCGGCAAGGAGGCGCCACACCCGAGGA										
	Section 17										
(1217) 1217		1230	1240	1250	1260	1270	1280				1292
GagMod.SF2(1217)	ACTGCCGCGGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCGCGGAGGCGCCACAGATGAAGGACTGCACCGAGCG										
GagProfMod.SF2(GP1)(1217)	ACTGCCGCGGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCGCGGAGGCGCCACAGATGAAGGACTGCACCGAGCG										
GagProfMod.SF2(GP2)(1217)	ACTGCCGCGGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCGCGGAGGCGCCACAGATGAAGGACTGCACCGAGCG										
Consensus(1217)	ACTGCCGCGGCCCCCGCAAGAAGGGCTGCTGGCGCTGCGGCGCGGAGGCGCCACAGATGAAGGACTGCACCGAGCG										
	Section 18										
(1293) 1293		1300	1310	1320	1330	1340	1350				1368
GagMod.SF2(1293)	CCAGGCCAACTTCTCCGCGCAAGATCTGGGCCAGCTACAAAGGCGCGCCCGGCAACTTCTTCAGAGCGCCCGGAG										
GagProfMod.SF2(GP1)(1293)	CCAGGCCAACTTCTCCGCGCAAGATCTGGGCCAGCTACAAAGGCGCGCCCGGCAACTTCTTCAGAGCGCCCGGAG										
GagProfMod.SF2(GP2)(1293)	CCAGGCCAACTTCTCCGCGCAAGATCTGGGCCAGCTACAAAGGCGCGCCCGGCAACTTCTTCAGAGCGCCCGGAG										
Consensus(1293)	CCAGGCCAACTTCTCCGCGCAAGATCTGGGCCAGCTACAAAGGCGCGCCCGGCAACTTCTTCAGAGCGCCCGGAG										
	Section 19										
(1369) 1369		1380	1390	1400	1410	1420	1430				1444
GagMod.SF2(1369)	CCACACGCGCGCGCGCGAGAGAGCTTCCGCTTCGGCGAGGAGAGAACACCCCGAGCCAGCAAGCAGGAGCCCATCG										
GagProfMod.SF2(GP1)(1369)	CCACACGCGCGCGCGCGAGAGAGCTTCCGCTTCGGCGAGGAGAGAACACACCCCGAGCCAGCAAGCAGGAGCCCATCG										
GagProfMod.SF2(GP2)(1369)	CCACACGCGCGCGCGCGAGAGAGCTTCCGCTTCGGCGAGGAGAGAACACACCCCGAGCCAGCAAGCAGGAGCCCATCG										
Consensus(1369)	CCACACGCGCGCGCGCGAGAGAGCTTCCGCTTCGGCGAGGAGAGAACACACCCCGAGCCAGCAAGCAGGAGCCCATCG										
	Section 20										
(1445) 1445		1450	1460	1470	1480	1490	1500	1510			1520
GagMod.SF2(1445)	ACAAAGGAGCTGTACCCCTGACCCAGCCCTGCGCAGCCCTGTTCGGCAACGACCCAGCAGCCAGTAA-----										
GagProfMod.SF2(GP1)(1445)	ACAAAGGAGCTGTGTACCCCTGACCCAGCCCTGCGCAGCCCTGTTCGGCAACGACCCCTGCTCAAGTAAAGGATCGGCGG										
GagProfMod.SF2(GP2)(1445)	ACAAAGGAGCTGTGTACCCCTGACCCAGCCCTGCGCAGCCCTGTTCGGCAACGACCCCTGCTCAAGTAAAGGATCGGCGG										
Consensus(1445)	ACAAAGGAGCTGTGTACCCCTGACCCAGCCCTGCGCAGCCCTGTTCGGCAACGACCCCTGCTCAAGTAAAGGATCGG										

FIG. 80D

Alignment GagMod vs GP1_GP2



TataminoSF162.opt

ATGGAGCCCGTGGACCCCGCCTGGAGCCCTGGAAGCACCCCGGCAGCCAGCCCAA
GACCGCCTGCACCAACTGCTACTGCAAGAAGTGCTGCTTCCACTGCCAGGTGTGCTT
CATCACCAAGGGCCTGGGCATCAGCTACGGCCGCAAGAAGCGCCGCCAGCGCCGC

FIG. 81 (SEQ ID NO:89)

660321" 57534760

0674

COMBINED DECLARATION AND POWER OF ATTORNEY
FOR UTILITY PATENT APPLICATION

AS A BELOW-NAMED INVENTOR, I HEREBY DECLARE THAT:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if more than one name is listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND PRODUCTION OF VIRUS-LIKE PARTICLES the specification of which

X is attached hereto
___ was filed on

and assigned Serial No. and was amended on .

I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

I acknowledge and understand that I am an individual who has a duty to disclose information which is material to the patentability of the claims of this application in accordance with Title 37, Code of Federal Regulations, §§ 1.56(a) and (b) which state:

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated

through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) prior art cited in search reports of a foreign patent office in a counterpart application, and

(2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or

(2) It refutes, or is inconsistent with, a position the applicant takes in:

(i) Opposing an argument of unpatentability relied on by the Office,

or

(ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

I do not know and do not believe this invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to said application. This invention was not in public use or on sale in the United States of America more than one year prior to this application. This invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on any application filed by me or my legal representatives or assigns more than six months prior to this application.

I hereby claim priority benefits under Title 35, United States Code § 119(e)(1) of any United States provisional application(s) for patent as indicated below and have also identified below any application for patent on this invention having a filing date before that of the application for patent on which priority is claimed:

<u>Application No.</u>	<u>Date of Filing</u> <u>(day/month/year)</u>	<u>Priority</u> <u>Claimed</u>
60/114,495	31 December 1998	Yes <u>X</u> No <u> </u>
60/168,471	01 December 1999	Yes <u>X</u> No <u> </u>

I hereby appoint the following attorneys and agents to prosecute that application and to transact all business in the Patent and Trademark Office connected therewith and to file, to prosecute and to transact all business in connection with all patent applications directed to the invention:

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Robert P. Blackburn, Reg. No. 30,447
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Joseph H. Guth, Reg. No. 31,261
Alisa A. Harbin, Reg. No. 33,895
Charlene A. Launer, Reg. No. 33,035
David P. Lentini, Reg. No. 33,944
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Vandana Date, Reg. No. 38,675
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Intellectual Property - R440
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Emeryville, CA 94662-8097.

Address all telephone calls to: Anne S. Dollard, Esq. at 510-923-2719.

This appointment, including the right to delegate this appointment, shall also apply to the same extent to any proceedings established by the Patent Cooperation Treaty.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Date _____

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Date _____

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Signature: _____

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 LIAN, Ying
 HARTOG, Karin
 LIU, Hong
 GREER, Catherine
 SELBY, Mark
 WALKER, Christopher

<120> IMPROVED EXPRESSION OF HIV POLYPEPTIDES AND PRODUCTION
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HIV-Gag

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<211> 1853

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
HIV-Gag-protease

<400> 5

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<211> 4319

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
HIV-Gag-polymerase

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<210> 7

<211> 2031

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic
HIV-Gag/HCV-core fusion polypeptide

<400> 7

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<210> 8
<211> 2025
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic
HIV-Gag/HCV-Core fusion polypeptide

<400> 8
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ctagaacgat tcgcagtcaa tcttggcctg ttagaaacat cagaaggctg cagacaaata 180
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<210> 9
<211> 1268
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic Gag
common region

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<400> 9
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cgcgagctgg agcgcttcgc cgtgaacccc ggctgctgg agaccagcga gggctgccgc 180
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ggccgcga 1268
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<210> 10

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: HIV-Gag
peptide p7G

<400> 10

Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu
1 5 10 15

Glu Ala Ala Glu
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<210> 11

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer GAG5

<400> 11

aagaattcca tgggtgagc agcgtcggtg

30

<210> 12
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: primer
 p55-SAL3

 <400> 12
 attcgtcgac tgtgacgagg ggtcgttgcc 30

 <210> 13
 <211> 34
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: primer
 CORESAL5

 <400> 13
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 <210> 14
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: primer 173CORE

 <400> 14
 tattggatcc taagagcaac caggaagggtt c 31

 <210> 15
 <211> 21
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: primer MS65

 <400> 15
 cgaccatcat ggatgcagcg c 21

 <210> 16
 <211> 30
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence: primer MS66

<400> 16
aggattcgtc gagtcgctgc tggggtcgtt 30

<210> 17
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer XPANXNF

<400> 17
gcacgtgggc ccggcgctc tagagc 26

<210> 18
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer XPANXNR

<400> 18
gctctagagg cgccgggccc acgtgc 26

<210> 19
<211> 20
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: HIV p55 Gag
Major Homology Region

<400> 19
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1 5 10 15

Phe Tyr Lys Thr
20

<210> 20
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: synthetic p55
Gag Major Homology Region

<400> 20
gacatccgcc agggcccaaa ggagcccttc cgcgactacg tggaccgctt ctacaagacc 60

<210> 21
 <211> 15
 <212> PRT
 <213> Human immunodeficiency virus

<400> 21
 Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg
 1 5 10 15

<210> 22
 <211> 5
 <212> PRT
 <213> Human immunodeficiency virus

<400> 22
 Lys Ala Lys Arg Arg
 1 5

<210> 23
 <211> 4
 <212> PRT
 <213> Human immunodeficiency virus

<400> 23
 Arg Glu Lys Arg
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<210> 24
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: aa of
 mut7.SF162 cleavage site

<400> 24
 Ala Pro Thr Lys Ala Ile Ser Ser Val Val Gln Ser Glu Lys Ser
 1 5 10 15

<210> 25
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: aa of
 mut8.SF162 cleavage site

<400> 25

Ala Pro Thr Ile Ala Ile Ser Ser Val Val Gln Ser Glu Lys Ser
 1 5 10 15

<210> 26
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: aa of
 mut.SF162 cleavage site

<400> 26
 Ala Pro Thr Lys Ala Lys Arg Arg Val Val Gln Arg Glu Lys Ser
 1 5 10 15

<210> 27
 <211> 15
 <212> PRT
 <213> Human immunodeficiency virus

<220>
 <223> Description of Artificial Sequence: aa of native
 cleavage site in US4

<400> 27
 Ala Pro Thr Gln Ala Lys Arg Arg Val Val Gln Arg Glu Lys Arg
 1 5 10 15

<210> 28
 <211> 5
 <212> PRT
 <213> Human immunodeficiency virus

<220>
 <223> Description of Artificial Sequence: aa of second
 cleavage site in US4

<400> 28
 Gln Ala Lys Arg Arg
 1 5

<210> 29
 <211> 15
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: aa of mut.US4
 cleavage site

<400> 29

Ala Pro Thr Gln Ala Lys Arg Arg Val Val Gln Arg Glu Lys Ser

1

5

10

15

<210> 30

<211> 1419

<212> DNA

<213> Human immunodeficiency virus

<400> 30

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<210> 31

<211> 1932

<212> DNA

<213> Human immunodeficiency virus

<400> 31

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<210> 32

<211> 2457

<212> DNA

<213> Human immunodeficiency virus

<400> 32

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gggagagcat tttatgcaac aggagacata ataggagata taagacaagc acattgtaac 900
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cacagtttta attgtggagg ggaatttttc tactgtaatt caacacagct ttttaatagt 1080
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gtacaggcca gacaattatt gtctgggtata gtgcaacagc agaacaattt gctgagagct 1560

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acaaacttaa tatacacctt aattgaagaa tcgcagaacc aacaagaaaa gaatgaacaa 1860
gaattattag aattggataa gtgggcaagt ttgtggaatt ggtttgacat atcaaaatgg 1920
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gctatagcag tagctgaggg gacagatagg attatagaag tagcacaag aattggtaga 2400
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```

<210> 33

<211> 1453

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp120.modSF162

<400> 33

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cccggtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcctacgac 180
accgaggtgc acaacgtgtg gggcaccac gctgtgctgc ccaccgacc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcactgcacc aacctgaaga acgcccacaa caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgaccacc 480
agcatccgca acaagatgca gaaggagtag gccctgttct acaagctgga cgtggtgccc 540
atcgacaacg acaacaccag ctacaagctg atcaactgca acaccagcgt gatcaccacg 600
gcctgcccc aagtgagctt cgagcccac cccatccact actgcgcccc cgccggcttc 660
gccatcctga agtgcaacga caagaagttc aacggcagcg gcccctgcac caacgtgagc 720
accgtgcagt gcaccacagg catccgcccc gtggtgagca ccagctgct gctgaacggc 780
agcctggccg aggagggcgt ggtgatccgc agcgagaact tcaccgacaa cgccaagacc 840
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ggcggcgacc ccgagatcgt gatgcacagc ttcaactgcg gcggcgagtt cttctactgc 1140
aacagcacc agctgttcaa cagcacctgg aacaacacca tcggcccaa caacaccaac 1200
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ggcggcgggc acatgcgcga caactggcgc agcgagctgt acaagtacaa ggtggtgaag 1440
atcgagcccc tgg 1453
```

<210> 34

<211> 1387

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp120.modSF162.delV2

<400> 34

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gcagtcttcg tttcgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgctg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
accgaggtgc acaacgtgtg ggccacccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcactgcacc aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgggcgcc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagg cctgccccaa ggtgagcttc 540
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ggcaaggaga tcagcaacac caccgagatc ttccgcccc gcggcggcga catgcgcgac 1320
aactggcgca gcgagctgta caagtacaag gtggtgaaga tcgagcccct gggcgtggcc 1380
cccacca
```

<210> 35

<211> 1323

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp120.modSF162.delV1V2

<400> 35

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
accgaggtgc acaacgtgtg ggccacccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgggcgc cggcaactgc cagaccagcg tgatcaccca ggccctgccc 420
aaggtgagct tcgagcccat ccccatccac tactgcgccc ccgcccggctt cgccatcctg 480
aagtgcacg acaagaagtt caacggcagc ggcccctgca ccaacgtgag caccgtgcag 540
tgcacccacg gcatccgccc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc 600
gaggagggcg tgggtgatccg cagcgagaac ttaccgaca acgccaagac catcatcgtg 660
cagctgaagg agagcgtgga gatcaactgc acccgcccc acaacaacac ccgcaagagc 720
```


aactggttcg acatcagcaa gtggctgtgg tacatctaac tcgag

2025

<210> 37

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.modSF162.delV2

<400> 37

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cccgtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcaa ggcctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgctgct ccaccgacct caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgacct gcactgcacc aacctgaaga acgccaccaa caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgggcgcc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagg cctgccccaa ggtgagcttc 540
gagcccatcc ccatccacta ctgcgcccc gccggcttcg ccatcctgaa gtgcaacgac 600
aagaagttca acggcgagcg cccctgcacc aacgtgagca ccgtgcagtg caccacggc 660
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tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggccc agcctgtgga actggttcga catcagcaag 1920
tggctgtggt acatctaact cgag 1944

<210> 38

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.modSF162.delV1/V2

<400> 38

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gcagtcttcg tttcgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
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gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggcc agcctgtgga actggttcga catcagcaag 1920
tggtgtggtt acatctaact cgag 1944

```

<210> 39

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modSF162

<400> 39

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcaactgcac aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgaccacc 480
agcatccgca acaagatgca gaaggagtac gccctgttct acaagctgga cgtggtgccc 540

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atcgacaacg acaacaccag ctacaagctg atcaactgca acaccagcgt gatcaccag 600
gcctgcccc aagtgagctt cgagcccac cccatccact actgcgcccc cgccggcttc 660
gccatcctga agtgcaacga caagaagttc aacggcagcg gcccctgcac caacgtgagc 720
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aaccagcagg agaagaacga gcaggagctg ctggagctgg acaagtgggc cagcctgtgg 1980
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```

<210> 40

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modSF162.delV2

<400> 40

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cccgtgtgga aggaggccac caccacctg ttctgcgcca gcgacgcaa ggcctacgac 180
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atgcacagct tcaactgcgg cggcgagttc ttctactgca acagcaccca gctgttcaac 1080

```

```

agcacctgga acaacacccat cggccccaac aacaccaacg gcaccatcac cctgcccctgc 1140
cgcacatcaagc agatcatcaa ccgctggcag gaggtgggca aggccatgta cgcaccccccc 1200
atccgcggcc agatccgctg cagcagcaac atcaccggcc tgctgctgac ccgcgacggc 1260
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tacaccaacc tgatctaac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggcc agcctgtgga actggttcga catcagcaag 1920
tggctgtggt acatctaact cgag 1944

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<210> 41

<211> 1836

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modSF162.delV1/V2

<400> 41

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcctacgac 180
accgaggtgc acaacgtgtg ggccacccac gcttgcgtgc ccaccgacc caacccccag 240
gagatcgtagc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgggcgc cggcaactgc cagaccagcg tgatcaccca ggccctgcccc 420
aaggtgagct tcgagcccat ccccatccac tactgcgccc ccgccggctt cgccatcctg 480
aagtgaacg acaagaagtt caacggcagc gggccctgca ccaacgtgag caccgtgcag 540
tgcaccacg gcatccgccc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc 600
gaggagggcg tggatgatccg cagcgagaac ttaccgaca acgccaagac catcatcgtg 660
cagctgaagg agagcgtgga gatcaactgc acccgcccca acaacaacac ccgcaagagc 720
atcaccatcg gcccggccg cgccttctac gccaccggcg acatcatcgg cgacatccgc 780
caggccact gcaacatcag cggcgagaag tggacaacaa ccctgaagca gatcgtgacc 840
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cccagatcgc tgatgcacag cttcaactgc ggcggcgagt tcttctactg caacagcacc 960
cagctgttca acagcacctg gaacaacacc atcgcccca acaacaccaa cggcaccatc 1020
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tacgcccccc ccatccgagg ccagatccgc tgcagcagca acatcaccgg cctgctgctg 1140
accgcgacg gcggcaagga gatcagcaac accaccgaga tcttcgccc cggcggcgcc 1200
gacatgcgcg acaactggcg cagcgagctg tacaagtaca aggtggtgaa gatcagccc 1260
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aagcagctgc agggccgct gctggccgtg gagcgtacc tgaaggacca gcagctgctg 1560
ggcatctggg gctgcagcgg caagctgatc tgcaaccacg ccgtgccctg gaacgccagc 1620
tggagcaaca agagcctgga ccagatctgg aacaacatga cctggatgga gtgggagcgc 1680

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gagatcgaca actacaccaa cctgatctac accctgatcg aggagagcca gaaccagcag 1740
gagaagaacg agcaggagct gctggagctg gacaagtggg ccagcctgtg gaactgggtc 1800
gacatcagca agtgggtgtg gtacatctaa ctcgag 1836

<210> 42
<211> 2025
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
gp140.mut7.modSF162

<400> 42
gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg tttcgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccggtgtgga aggagggcac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gactgcacc aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgaccacc 480
agcatccgca acaagatgca gaaggagtac gccctgttct acaagctgga cgtggtgccc 540
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gcctgccccca aggtgagctt cgagcccact cccatccact actgcgcccc cgccggcttc 660
gccatcctga agtgcaacga caagaagttc aacggcagcg gccctgcac caacgtgagc 720
accgtgcagt gcacccaagg catccgcccc gtggtgagca ccagctgct gctgaacggc 780
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gcgaagagca tcaccatcgg ccccgccgc gccttctacg ccaccggcga catcatcggc 960
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aacagcacc agctgttcaa cagcacctgg aacaacacca tcggcccaa caacaccaac 1200
ggcaccatca ccctgccctg ccgcatcaag cagatcatca accgctggca ggaggtgggc 1260
aaggccatgt acgcccccc catccgcggc cagatccgct gcagcagcaa catcaccggc 1320
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cagcagaaca acctgctgcg gcgcatcgag gcccagcagc acctgctgca gctgaccgtg 1680
tggggcatca agcagctgca ggcccgcgtg ctggccgtgg agcgctacct gaaggaccag 1740
cagctgctgg gcatctgggg ctgcagcggc aagctgatct gcaccaccgc cgtgccctgg 1800
aacgccagct ggagcaacaa gagcctggac cagatctgga acaacatgac ctggatggag 1860
tgggagcgcg agatcgacaa ctacaccaac ctgatctaca ccctgatcga ggagagccag 1920
aaccagcagg agaagaacga gcaggagctg ctggagctgg acaagtgggc cagcctgtgg 1980
aactggttcg acatcagcaa gtggctgtgg tacatctaac tcgag 2025

<210> 43
<211> 1944
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut7.modSF162.delV2

<400> 43

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gcactgcacc aacctgaaga acgccaccaa caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgggcgcc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagc cctgccccaa ggtgagcttc 540
gagcccatcc ccatccacta ctgcgcccc gccggcttcg ccatcctgaa gtgcaacgac 600
aagaagttca acggcagcgg cccctgcacc aacgtgagca ccgtgcagtg caccacggc 660
atccgccccg tgggtgagcac ccagctgctg ctgaacggca gcctggccga ggagggcgtg 720
gtgatccgca gcgagaactt caccgacaac gccaagacca tcatcgtgca gctgaaggag 780
agcgtggaga tcaactgcac ccgccccaac aacaacaccc gcaagagcat caccatcggc 840
ccggcgccg ccttctacgc caccggcgac atcatcggcg acatccgcca ggcccactgc 900
aacatcagcg gcgagaagtg gaacaacacc ctgaagcaga tcgtgaccaa gctgcaggcc 960
cagttcggca acaagaccat cgtgttcaag cagagcagcg gcggcgaccc cgagatcgtg 1020
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cgcacaaagc agatcatcaa ccgctggcag gaggtgggca aggccatgta ccccccccc 1200
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tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggccc agcctgtgga actggttcga catcagcaag 1920
tggctgtggt acatctaact cgag 1944

<210> 44

<211> 1836

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut7.modSF162.delV1/V2

<400> 44

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcctacgac 180
accgaggtgc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300

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cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtggggcg cggaactgc cagaccagcg tgatcaccca ggcctgcccc 420
aaggtgagct tcgagcccat ccccatccac tactgcgccc ccgcccggctt cgccatcctg 480
aagtgaacg acaagaagtt caacggcagc gggccctgca ccaacgtgag caccgtgcag 540
tgcaccacg gcatccgccc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc 600
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cagctgaagg agagcgtgga gatcaactgc acccgcccca acaacaacac ccgcaagagc 720
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gacatgcgcg acaactggcg cagcgagctg tacaagtaca aggtggtgaa gatcgagccc 1260
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agcctgaccc tgaccgtgca ggcccggcag ctgctgagcg gcacgtgca gcagcagaac 1440
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aagcagctgc agggccgctg gctggccgtg gagcgctacc tgaaggacca gcagctgctg 1560
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tgagcaaca agagcctgga ccagatctgg aacaacatga cctggatgga gtgggagcgc 1680
gagatcgaca actacaccaa cctgatctac accctgatcg aggagagcca gaaccagcag 1740
gagaagaacg agcaggagct gctggagctg gacaagtggg ccagcctgtg gaactggttc 1800
gacatcagca agtggctgtg gtacatctaa ctcgag 1836

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<210> 45

<211> 2025

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut8.modSF162

<400> 45

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcctacgac 180
accgaggtgc acaactgtgt ggccacccac gcctgctgct ccaccgaccc caacccccag 240
gagatcgtgc tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgacctt gcaactgcacc aacctgaaga acgccaccaa caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgaccacc 480
agcatccgca acaagatgca gaaggagtac gccctgttct acaagctgga cgtggtgccc 540
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gctgccccca aggtgagctt cgagcccacat cccatccact actgcgcccc cgccggcttc 660
gccatcctga agtgcaacga caagaagtcc aacggcagcg gccctgcac caactgtgagc 720
accgtgcagt gcacccacgg catccgcccc gtggtgagca ccagctgct gctgaacggc 780
agcctggccg aggagggcgt ggtgatccgc agcgagaact tcaccgacaa cgccaagacc 840
atcatcgtgc agctgaagga gagcgtggag atcaactgca cccgccccaa caacaacacc 900
cgcaagagca tcaccatcgg ccccgccgcg gccttctacg ccaccggcga catcatcggc 960
gacatccgcc agggccactg caacatcagc ggcgagaagt ggaacaacac cctgaagcag 1020

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atcgtgacca agctgcaggc ccagttcggc aacaagacca tcgtgttcaa gcagagcagc 1080
ggcggcgacc ccgagatcgt gatgcacagc ttcaactgcg gcggcgagtt cttctactgc 1140
aacagcacc ccagctgttcaa cagcacctgg aacaacacca tcggcccca caacaccaac 1200
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aaggccatgt acgccccccc catccgcggc cagatccgct gcagcagcaa catcaccggc 1320
ctgctgctga cccgcgacgg cggcaaggag atcagcaaca ccaccgagat cttccgcccc 1380
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atcgagcccc tgggcgtggc cccaccatc gccatcagca gcgtggtgca gagcgagaag 1500
agcgccgtga ccctggggcg catgttcttg ggcttctctg gcgcgcggc cagcaccatg 1560
ggcggccgca gcctgacct gaccgtgcag gcccgccagc tgctgagcgg catcgtgcag 1620
cagcagaaca acctgctgcg cgccatcgag gcccgagcgc acctgctgca gctgaccgtg 1680
tggggcatca agcagctgca ggcccgctg ctggccgtgg agcgctacct gaaggaccag 1740
cagctgctgg gcatctggg ctgcagcggc aagctgatct gcaccaccgc cgtgccctgg 1800
aacgcagct ggagcaacaa gagcctggac cagatctgga acaacatgac ctggatggag 1860
tgggagcgcg agatcgacaa ctacaccaac ctgatctaca ccctgatcga ggagagccag 1920
aaccagcagg agaagaacga gcaggagctg ctggagctgg acaagtgggc cagcctgtgg 1980
aactggttcg acatcagcaa gtggctgtgg tacatctaac tcgag 2025

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<210> 46

<211> 1944

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut8.modSF162.delV2

<400> 46

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gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg ttctgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgctg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggccctacgac 180
accgaggtgc acaactgtgtg ggccaccac gcctgctgct ccaccgacc caacccccag 240
gagatcgctg tggagaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgacct gcaactgcacc aacctgaaga acgccacca caccaagagc 420
agcaactgga aggagatgga ccgcggcgag atcaagaact gcagcttcaa ggtgggcgcc 480
ggcaagctga tcaactgcaa caccagcgtg atcaccagg cctgccccaa ggtgagcttc 540
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gtgatccgca gcgagaactt caccgacaac gccaagacca tcatcgtgca gctgaaggag 780
agcgtggaga tcaactgcac ccgccccaac aacaacacc gcaagagcat caccatcggc 840
cccgcccgcg ccttctacgc caccggcgac atcatcggc acatccgcca ggcccactgc 900
aacatcagcg gcgagaagtg gaacaacacc ctgaagcaga tcgtgaccaa gctgcaggcc 960
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agcacctgga acaacacat ccggcccaac aacaccaac gcaccatcac cctgccctgc 1140
cgcacatcagc agatcatcaa ccgctggcag gaggtgggca aggccatgta cgccccccc 1200
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cccaccatcg ccatcagcag cgtggtgcag agcgagaaga gcgcgctgac cctgggcgcc 1440
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gccatcgagg cccagcagca cctgctgcag ctgaccgtgt ggggcatcaa gcagctgcag 1620
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tacaccaacc tgatctacac cctgatcgag gagagccaga accagcagga gaagaacgag 1860
caggagctgc tggagctgga caagtgggcc agcctgtgga actggttcga catcagcaag 1920
tggctgtggt acatctaact cgag                                     1944

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<210> 47

<211> 1836

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp140.mut8.modSF162.delV1/V2

<400> 47

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gcagtcttcg tttcgcccag cgccgtggag aagctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcctacgac 180
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cagatgcacg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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aaggtgagct tcgagcccat ccccatccac tactgcgccc ccgccggctt cgccatcctg 480
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tgcaccacg gcatccgccc cgtggtgagc acccagctgc tgctgaacg cagcctggcc 600
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caggcccaact gcaacatcag cggcgagaag tggaaacaac ccctgaagca gatcgtgacc 840
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cccgagatcg tgatgcacag cttcaactgc ggcggcgagt tcttctactg caacagcacc 960
cagctgttca acagcacctg gaacaacacc atcgccccca acaacaccaa cggcaccatc 1020
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gagaagaacg agcaggagct gctggagctg gacaagtggg ccagcctgtg gaactggttc 1800
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```

<210> 48

<211> 2547

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp160.modSF162

<400> 48

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gaggtggccc agcgcacgag ccgcgccttc ctgcacatcc cccgccgcat ccgccagggc 2520
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<210> 49

<211> 2466

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp160.modSF162.delV2

<400> 49

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<210> 50

<211> 2358

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp160.modSF162.delV1/V2

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gccctgctgt aactcag 2358

<210> 51

<211> 1494

<212> DNA

<213> Human immunodeficiency virus

<400> 51

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acacatgcct gtgtaccac agacccaac ccacaggaag taaatttaac aaatgtgaca 180
gaaaatttta acatgtggaa aaataacatg gtggaacaga tgcagagga tataatcagt 240

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gaatattctc tcttctataa acttgatgta gtaccaatag ataatgataa tgctagctat 540
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<210> 52

<211> 2007

<212> DNA

<213> Human immunodeficiency virus

<400> 52

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<210> 53
<211> 2532
<212> DNA
<213> Human immunodeficiency virus
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tggagtaata aatctctgac tgagatttgg gataatatga cctggatgga gtgggaaaga 1860
gaaattggca attatacagg cttaatatatac aatttaattg aaatagcaca aaaccagcaa 1920
gaaaagaatg aacaagaatt attggaatta gacaagtggg caagtttgtg gaattggttt 1980
gatataacaa actggctgtg gtatataaga atattcataa tgatagtagg aggttgata 2040
ggtttaagaa tagtttttgc tgtactttct atagtgaata gagttaggca gggatactca 2100
ccaatatcat tgcagaccg cctccagct cagaggggac ccgacaggcc cgaaggaatc 2160
gaagaagaag gtggagagag agacagagac agatccaatc gattagtgc tggattattg 2220
gcactcatct gggacgatct gcggagcctg tgctcttca gctaccaccg cttgagagac 2280
ttactcttga ttgtagcgag gattgtggaa cttctgggac gcaggggggtg ggaagccctc 2340
```

```

aagtattggt ggaatctcct gcagtattgg agtcaggagc taaagagtag tgctgttagt 2400
ttgtttaatg ccacagcaat agcagtagct gaagggacag ataggattat agaaatagta 2460
caaagaattt ttagagctgt aattcacata cctagaagaa taagacaggg cttggagagg 2520
gctttactat aa 2532

```

```

<210> 54
<211> 1599
<212> DNA
<213> Artificial Sequence

```

```

<220>
<223> Description of Artificial Sequence: gp120.modUS4

```

```

<400> 54
gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg tttcgcccag cgccaccacc gtgctgtggg tgaccgtgta ctacggcgtg 120
cccggtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gaactgcacc gacaagctga ccggcagcac caacggcacc 420
aacagcacca gcggcaccaa cagcaccagc ggcaccaaca gcaccagcac caacagcacc 480
gacagctggg agaagatgcc cgagggcgag atcaagaact gcagcttcaa catcaccacc 540
agcgtgcgcg acaaggtgca gaaggagtac agcctgttct acaagctgga cgtggtgccc 600
atcgacaacg acaacgccag ctaccgcctg atcaactgca acaccagcgt gatcaccacg 660
gcctgccccca aggtgagctt cgagcccact cccatccact actgcgcccc cgccggcttc 720
gccatcctga agtgcaagga caagaagttc aacggcaccg gccctgcaa gaacgtgagc 780
accgtgcagt gcacccacgg catccgcccc gtggtgagca ccagctgct gctgaacggc 840
agcctggccg aggaggagat cgtgctgcgc tccgagaact tcaccgacaa cgccaagacc 900
atcatcgtgc agctgaacga gtccgtggag atcaactgca tccgccccaa caacaacacg 960
cgtaagagca tccacatcgg ccccgccgc gccttctacg ccaccggcga catcatcggc 1020
gacatccgcc aggccactg caacatcagc aaggccaact ggaccaacac cctcgagcag 1080
atcgtggaga agctgcgcga gcagttcggc aacaacaaga ccatcatctt caacagcagc 1140
agcggcgccg accccgagat cgtgttccac agcttcaact gcggcgccga gttctttctac 1200
tgcaacacca gccagctgtt caacagcacc tggaacatca ccgaggaggt gaacaagacc 1260
aaggagaacg acaccatcat cctgccttgc cgcattccgc agatcatcaa catgtggcag 1320
gaggtgggca aggccatgta ccccccccc atccgcggcc agatcaagtg cagcagcaat 1380
attaccggcc tgctgtgac ccgcgacggc ggcaccaaca acaaccgcac caacgacacc 1440
gagaccttcc gcccggcgcg cggcaacatg aaggacaact ggcgcagcga gctgtacaag 1500
tacaaggtgg tgcgcatcga gcccctgggc gtggccccc cccaggccaa gcgccgctg 1560
gtgcagcgcg agaagcgcta agatatcgga tcctctaga 1599

```

```

<210> 55
<211> 1350
<212> DNA
<213> Artificial Sequence

```

```

<220>
<223> Description of Artificial Sequence:
      gp120.modUS4.del 128-194

```

```

<400> 55
gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg tttcgcccag cgccaccacc gtgctgtggg tgaccgtgta ctacggcgtg 120

```



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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
gccgaggccc acaacgtgtg ggccacccac gcctgcgtgc ccaccgaccc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgggggc agggaaactgc gagaccagcg tgatcaccca ggcttgcccc 420
aagtgagct tcgagcccat ccccatccac tactgcgccc ccgcccggctt cgccatcctg 480
aagtgaagg acaagaagtt caacggcacc ggcccctgca agaacgtgag caccgtgcag 540
tgcaccacg gcatccgccc cgtggtgagc acccagctgc tgctgaacgg cagcctggcc 600
gaggaggaga tcgtgctgcg ctccgagaac ttaccgaca acgccaagac catcatcgtg 660
cagctgaacg agtcctgtga gatcaactgc atccgcccc acaacaacac gcgtaagagc 720
atccacatcg gccccggcg cgcttctac gccaccggcg acatcatcgg cgacatccgc 780
caggcccact gcaacatcag caaggccaac tggaccaaca ccctcgagca gatcgtggag 840
aagctgcgag agcagttcgg caacaacaag accatcatct tcaacagcag cagcggcggc 900
gaccccgaga tcgtgttcca cagcttcaac tgcggcggcg agttcttcta ctgcaacacc 960
agccagctgt tcaacagcac ctggaacatc accgaggagg tgaacaagac caaggagaac 1020
gacaccatca tcttgccctg ccgcatccgc cagatcatca acatgtggca ggaggtgggc 1080
aaggccatgt acgccccccc catccgcggc cagatcaagt gcagcagcaa tattaccggc 1140
ctgctgctga cccgcgacgg cggcaccaac aacaaccgca ccaacgacac cgagaccttc 1200
cgccccggcg gcggaacat gaaggacaac tggcgagcg agctgtacaa gtacaagggtg 1260
gtgcgcatcg agcccctggg cgtggccccc acccaggcca agcgccgcgt ggtgcagcgc 1320
gagaagcgct aagatatcgg atcctctaga                                     1350

```

<210> 56

<211> 2112

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp140.modUS4

<400> 56

```

gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg tttcgcccag cgccaccacc gtgctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
gccgaggccc acaacgtgtg ggccacccac gcctgcgtgc ccaccgaccc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gaactgcacc gacaagctga ccggcagcac caacggcacc 420
aacagacca gcggcaccaa cagcaccagc ggcaccaaca gcaccagcac caacagcacc 480
gacagctggg agaagatgcc cgaggggcag atcaagaact gcagcttcaa catcaccacc 540
agcgtgcgag acaagggtgca gaaggagtac agcctgttct acaagctgga cgtggtgccc 600
atcgacaacg acaacgccag ctaccgcctg atcaactgca acaccagcgt gatcaccag 660
gcctgcccc aagtgagctt cgagcccact cccatccact actgcgcccc cgccggcttc 720
gccatcctga agtgcaagga caagaagttc aacggcaccg gcccctgcaa gaacgtgagc 780
accgtgcagt gcaccacagg catccgcccc gtggtgagca ccagctgct gctgaacggc 840
agcctggccg aggaggagat cgtgctgcgc tccgagaact tcaccgaaa cgccaagacc 900
atcatcgtgc agtgaaacga gtccgtggag atcaactgca tccgccccaa caacaacacg 960
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gacatccgcc aggccactg caacatcagc aaggccaact ggaccaacac cctcgagcag 1080
atcgtggaga agctgcgcga gcagttcggc aacaacaaga ccatcatctt caacagcagc 1140
agcggcggcg accccgagat cgtgttccac agcttcaact gcggcggcga gttcttctac 1200
tgcaacacca gccagctgtt caacagcacc tggaaacatca ccgaggaggt gaacaagacc 1260
aaggagaacg acaccatcat cctgccctgc cgcacccgcc agatcatcaa catgtggcag 1320
gaggtgggca aggccatgta cgcccccccc atcccgcgcc agatcaagtg cagcagcaat 1380

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attaccggcc tgctgctgac ccgcgacggc ggcaccaaca acaaccgcac caacgacacc 1440
gagaccttcc gccccggcgg cggcaacatg aaggacaact ggcgcagcga gctgtacaag 1500
tacaaggtgg tgcgcatcga gcccctgggc gtggccccc cccaggccaa gcgccgcgtg 1560
gtgcagcgcg agaagcgcg cgtgggcctg ggcgcctgt tcatcggctt cctgggcgcc 1620
gccgggagca ccatgggcgc cgctccgtg accctgaccg tgcaggcccc ccagctgctg 1680
agcggcatcg tgcagcagca gaacaacctg ctgcgcgcca tcgaggccca gcagcacctg 1740
ctgcagctga ccgtgtgggg catcaagcag ctgcaggccc gcctcctggc cgtggagcgc 1800
tacctgaagg accagcagct gctgggcatc tggggctgca gcggaagct gatctgcacc 1860
accaccgtgc cctggaacag cagctggagc aacaagagcc tgaccgagat ctgggacaac 1920
atgacctgga tggagtggga gcgcgagatc ggcaactaca ccggcctgat ctacaacctg 1980
atcgagatcg ccagaacca gcaggagaag aacgagcagg agctgctgga gctggacaag 2040
tgggccagcc tgtggaactg gttcgacatc accaactggc tgtggtacat ctaagatatc 2100
ggatcctcta ga                                     2112

```

<210> 57

<211> 2112

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modUS4

<400> 57

```

gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg tttcgcccag cgccaccacc gtgctgtggg tgaccgtgta ctacggcgtg 120
cccggtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccac gctgctgtgc ccaccgaccc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgacct gaactgcacc gacaagctga ccggcagcac caacggcacc 420
aacagcacca gcggcaccaa cagcaccagc ggcaccaaca gcaccagcac caacagcacc 480
gacagctggg agaagatgcc cgagggcgag atcaagaact gcagcttcaa catcaccacc 540
agcgtgcgcg acaagggtgca gaaggagtac agcctgttct acaagctgga cgtggtgccc 600
atcgacaacg acaacgccag ctaccgcctg atcaactgca acaccagcgt gatcaccag 660
gcctgcccga aggtgagctt cgagcccac cccatccact actgcgcccc cgccggcttc 720
gccatcctga agtgcaagga caagaagttc aacggcaccg gccctgcaa gaacgtgagc 780
accgtgcagt gcacccacgg catccgcccc gtggtgagca cccagctgct gctgaacggc 840
agcctggccg aggaggagat cgtgctgcgc tccgagaact tcaccgacaa cgccaagacc 900
atcatcgtgc agctgaacga gtccgtggag atcaactgca tccgcccga caacaacacg 960
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gacatccgcc agggccactg caacatcagc aaggccaact ggaccaacac cctcgagcag 1080
atcgtggaga agctgcgcga gcagttcggc aacaacaaga ccatcatctt caacagcagc 1140
agcggcggcg accccgagat cgtgttccac agcttcaact gcggcggcga gttcttctac 1200
tgcaacacca gccagctgtt caacagcacc tggaaacatca ccgaggaggt gaacaagacc 1260
aaggagaacg acaccatcat cctgcccctg cgcatccgcc agatcatcaa catgtggcag 1320
gaggtgggca aggccatgta cgcccccccc atccgcggcc agatcaagtg cagcagcaat 1380
attaccggcc tgctgctgac ccgcgacggc ggcaccaaca acaaccgcac caacgacacc 1440
gagaccttcc gccccggcgg cggcaacatg aaggacaact ggcgcagcga gctgtacaag 1500
tacaaggtgg tgcgcatcga gcccctgggc gtggccccc cccaggccaa gcgccgcgtg 1560
gtgcagcgcg agaagagcgc cgtgggcctg ggcgcctgt tcatcggctt cctgggcgcc 1620
gccgggagca ccatgggcgc cgctccgtg accctgaccg tgcaggcccc ccagctgctg 1680
agcggcatcg tgcagcagca gaacaacctg ctgcgcgcca tcgaggccca gcagcacctg 1740
ctgcagctga ccgtgtgggg catcaagcag ctgcaggccc gcctcctggc cgtggagcgc 1800

```

```
tacctgaagg accagcagct gctgggcatc tggggctgca gcggaagct gatctgcacc 1860
accaccgtgc cctggaacag cagctggagc aacaagagcc tgaccgagat ctgggacaac 1920
atgacctgga tggagtggga gcgcgagatc ggcaactaca ccggcctgat ctacaacctg 1980
atcgagatcg cccagaacca gcaggagaag aacgagcagg agctgctgga gctggacaag 2040
tgggccagcc tgtggaactg gttcgacatc accaactggc tgtggtacat ctaagatatc 2100
ggatcctcta ga 2112
```

<210> 58

<211> 2181

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp140TM.modUS4

<400> 58

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gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
gcagtcttcg tttcgcccag cgccaccacc gtgctgtggg tgaccgtgta ctacggcgtg 120
cccggtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccac gctgcgtgc ccaccgaccc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
acccccctgt gcgtgaccct gaactgcacc gacaagctga ccggcagcac caacggcacc 420
aacagcacca gcggcaccaa cagcaccagc ggcaccaaca gcaccagcac caacagcacc 480
gacagctggg agaagatgcc cgagggcgag atcaagaact gcagcttcaa catcaccacc 540
agcgtgcgcg acaaggtgca gaaggagtac agcctgttct acaagctgga cgtggtgccc 600
atcgacaacg acaacgccag ctaccgcctg atcaactgca acaccagcgt gatcaccag 660
gcctgcccc aagtgagctt cgagcccac cccatccact actgcgcccc cgccggcttc 720
gccatcctga agtgcaagga caagaagttc aacggcaccg gcccctgcaa gaacgtgagc 780
accgtgcagt gcaccacagg catccgcccc gtggtgagca cccagctgct gctgaacggc 840
agcctggcgg aggaggagat cgtgctgcgc tccgagaact tcaccgacaa cgccaagacc 900
atcatcgtgc agctgaacga gtccgtggag atcaactgca tccgccccaa caacaacacg 960
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atcgtggaga agctgcgcga gcagttcggc aacaacaaga ccatcatctt caacagcagc 1140
agcggcggcg accccgagat cgtgttccac agcttcaact gcggcggcga gttcttctac 1200
tgcaacacca gccagctgtt caacagcacc tggaacatca ccgaggaggt gaacaagacc 1260
aaggagaacg acaccatcat cctgccctgc cgcacccgcc agatcatcaa catgtggcag 1320
gaggtgggca aggccatgta cggccccccc atccgcggcc agatcaagtg cagcagcaat 1380
attaccggcc tgctgctgac ccgcgacggc ggcaccaaca acaaccgcac caacgacacc 1440
gagaccttcc gccccggcgg cggcaacatg aaggacaact ggcgcagcga gctgtacaag 1500
tacaaggtgg tgcgcatcga gcccctgggc gtggccccc cccaggccaa gcgccgctg 1560
gtgcagcgcg agaagcgcgc cgtgggcctg ggcgcctgt tcatcggtt cctgggcgcc 1620
gccgggagca ccatgggcgc cgctccgtg accctgaccg tgcaggccc ccagctgctg 1680
agcggcatcg tgcagcagca gaacaacctg ctgcgcgcca tcgaggcca gcagacctg 1740
ctgcagctga ccgtgtggg catcaagcag ctgcaggccc gcacccctggc cgtggagcgc 1800
tacctgaagg accagcagct gctgggcatc tggggctgca gcggaagct gatctgcacc 1860
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atcgagatcg cccagaacca gcaggagaag aacgagcagg agctgctgga gctggacaag 2040
tgggccagcc tgtggaactg gttcgacatc accaactggc tgtggtacat ccgcatcttc 2100
atcatgatcg tggcgccct gatcggcctg cgcacgtgt tgcgctgct gagcatcgtg 2160
taagatatcg gatcctctag a 2181
```

<210> 59
 <211> 1818
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 gp140.modUS4.delV1/V2

<400> 59
 gaattcgcca ccatggatgc aatgaagaga gggctctgct gtgtgctgct gctgtgtgga 60
 gcagtcttcg ttctgcccag cgccaccacc gtgctgtggg tgaccgtgta ctacggcgctg 120
 cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
 gccgaggccc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
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 cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgggcgcc 360
 ggccaggcct gcccgaaggt gagcttcgag cccatcccca tccactactg cgcccccgcc 420
 ggcttcgcca tcctgaagtg caaggacaag aagttcaacg gcaccggccc ctgcaagaac 480
 gtgagcaccg tgcagtgcac ccacggcatc cgccccgtgg tgagcaccga gctgctgctg 540
 aacggcagcc tggccgagga ggagatcgtg ctgcgctccg agaacttcac cgacaacgcc 600
 aagaccatca tcgtgcagct gaacgagtcc gtggagatca actgcatccg cccaacaac 660
 aacacgcgta agagcatcca catcgcccc ggccgcgcct tctacgccac cggcgacatc 720
 atcggcgaca tccgccaggc ccactgcaac atcagcaagg ccaactggac caacaccctc 780
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 ttctactgca acaccagcca gctgttcaac agcacctgga acatcaccga ggaggtgaac 960
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 agcaatatta cgggcctgct gctgacccgc gacggcggca ccaacaacaa ccgcaccaac 1140
 gacaccgaga ccttcgcgcc cggcgggcggc aacatgaagg acaactggcg cagcgagctg 1200
 tacaagtaca aggtggtgcg catcgagccc ctgggctggtg cccccacca ggccaagcgc 1260
 cgcgtggtgc agcgcgagaa gcgcgccgtg ggccgtggcg cctgtttcat cggcttctc 1320
 ggcgccgccc ggagcaccat gggcgccgcc tccgtgaccc tgaccgtgca ggcccgcag 1380
 ctgctgagcg gcatcgtgca gcagcagaac aacctgctgc gcgccatcga ggcccagcag 1440
 cacctgctgc agctgaccgt gtggggcatc aagcagctgc agggccgcat cctggccgtg 1500
 gagcgctacc tgaaggacca gcagctgctg ggcattctggg gctgcagcgg caagctgatc 1560
 tgcaccacca ccgtgccctg gaacagcagc tggagcaaca agagcctgac cgagatctgg 1620
 gacaacatga cctggatgga gtgggagcgc gagatcggca actacaccgg cctgatctac 1680
 aacctgatcg agatcgccca gaaccagcag gagaagaacg agcaggagct gctggagctg 1740
 gacaagtggg ccagcctgtg gaactggttc gacatcacca actggctgtg gtacatctaa 1800
 gatatcggat cctctaga 1818

<210> 60
 <211> 2031
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 gp140.modUS4.delV2

<400> 60
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 gcagtcttcg ttctgcccag cgccaccacc gtgctgtggg tgaccgtgta ctacggcgctg 120

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccac gcctgctg gcaccgaccc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
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aacagcacca gcggcaccaa cagcaccagc ggcaccaaca gcaccagcac caacagcacc 480
gacagctggg agaagatgcc cgaggcgag atcaagaact gcagcttcaa catcggcgcc 540
ggccgctga tcaactgcaa caccagcgtg atcaccagc cctgccccaa ggtgagcttc 600
gagcccatcc ccatccacta ctgccccccc gccggcttcg ccatcctgaa gtgcaaggac 660
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atccgccccg tggtagcac ccagctgctg ctgaacggca gcctggccga ggaggagatc 780
gtgctgcgt ccgagaactt caccgacaac gccaaagacca tcatcgtgca gctgaacgag 840
tccgtggaga tcaactgcat ccgccccaac aacaacacgc gtaagagcat ccacatcggc 900
cccgcccgcg ccttctacgc caccggcgac atcatcggcg acatccgcca ggcccactgc 960
aacatcagca aggccaaactg gaccaaacacc ctgcagcaga tcgtggagaa gctgcgcgag 1020
cagttcggca acaacaagac catcatcttc aacagcagca gcggcgccga ccccgagatc 1080
gtgttccaca gcttcaactg cggcgcgag ttcttctact gcaacaccag ccagctgttc 1140
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caggagaaga acgagcagga gctgctggag ctggacaagt gggccagcct gtggaactgg 1980
ttcgacatca ccaactggct gtggtacatc taagatatcg gatcctctag a 2031

```

<210> 61

<211> 1818

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modUS4.delV1/V2

<400> 61

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
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cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtggcgcc 360
ggccaggcct gcccaagggt gagcttcgag cccatcccca tccactactg cggccccgcc 420
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gtgagcaccg tgcatgacac ccacggcatc cgccccgtgg tgagcaccga gctgctgctg 540
aacggcagcc tggccgagga ggagatcgtg ctgcgctccg agaacttcac cgacaacgcc 600
aagaccatca tcgtgcagct gaacgagtc gtggagatca actgcatccg ccccaacaac 660

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aacacgcgta agagcatcca catcgggccc ggccgcgcct tctacgccac cggcgacatc 720
atcggcgaca tccgccaggc ccaactggac atcagcaagg ccaactggac caacaccctc 780
gagcagatcg tggagaagct gcgcgagcag ttcggcaaca acaagaccat catcttcaac 840
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gacaagtggg ccagcctgtg gaactggttc gacatcacca actggctgtg gtacatctaa 1800
gatatcggat cctctaga                                     1818

```

<210> 62

<211> 1818

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.modUS4.del 128-194

<400> 62

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gcagtcttcg tttcgcccag cgccaccacc gtgctgtggg tgaccgtgta ctacggcgtg 120
cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccacc gcctgcgtgc ccaccgaccc caacccccag 240
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cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgggcgcc 360
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aagaccatca tcgtgcagct gaacgagtcg gtggagatca actgcatccg cccaacaac 660
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tacaagtaca aggtggtgcg catcgagccc ctgggcgtgg ccccccacca ggccaagcgc 1260
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```

```

ctgctgagcg gcacgtgca gcagcagaac aacctgctgc gcgccatcga ggcccagcag 1440
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gagcgctacc tgaaggacca gcagctgctg ggcatctggg gctgcagcgg caagctgatc 1560
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gacaagtggg ccagcctgtg gaactggttc gacatcacca actggctgtg gtacatctaa 1800
gatatcggat cctctaga                                     1818

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<210> 63

<211> 1863

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp140.mut.modUS4.del 128-194

<400> 63

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccac gcctgcgtgc ccaccgaccc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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aaggtgagct tgcagcccat ccccatccac tactgcgccc ccgccggtt cgccatcctg 480
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cagctgaacg agtccgtgga gatcaactgc atccgcccc acaacaacac gcgtaagagc 720
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gccagaacc agcaggagaa gaacgagcag gagctgctgg agctggacaa gtgggcccagc 1800
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aga

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<210> 64

<211> 2634

<212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: gp160.modUS4

<400> 64

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
gccgaggccc acaacgtgtg ggccaccac gctgctgctg ccaccgacc caacccccag 240
gaggtgaacc tgaccaacgt gaccgagaac ttcaacatgt ggaagaacaa catggtggag 300
cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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aacagcacca gcggcaccia cagcaccagc ggaccaaca gcaccagcac caacagcacc 480
gacagctggg agaagatgcc cgaggcgag atcaagaact gcagcttcaa catcaccacc 540
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<210> 65

<211> 2538

<212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
 gp160.modUS4.delV1

<400> 65

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcca ggcttacaag 180
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cagatgcatg aggacatcat cagcctgtgg gaccagagcc tgaagccctg cgtgaagctg 360
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<210> 66

<211> 2553

<212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 gp160.modUS4.delV2

<400> 66
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 cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
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<210> 67
 <211> 2340

<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp160.modUS4.delV1/V2

<400> 67

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
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gagggcaccg accgcatcat cgagatcgtg cagcgcatct tccgcgccgt gatccacatc 2280
ccccgccgca tccgccaggg cctggagcgc gccctgctgt aagatatcgg atcctctaga 2340
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<210> 68

<211> 2385

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
gp160.modUS4del 128-194

<400> 68

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cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
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aaggtgagct tcgagcccat ccccatccac tactgcgccc ccgcccgtt cgccatcctg 480
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```

<210> 69

<211> 144

<212> DNA

<213> Human immunodeficiency virus

<400> 69

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aaggccatgt acgccccccc catccgcggc cagatcaagt gcagcagcaa catcaccggc 120
ctgctgctga cccgcgacgg cggc 144

```

<210> 70
 <211> 144
 <212> DNA
 <213> Human immunodeficiency virus

<400> 70
 ggaactatca cactcccatg cagaataaaa caaattataa acaggtggca ggaagtagga 60
 aaagcaatgt atgcccctcc catcagagga caaattagat gtcacatcaa tattacagga 120
 ctgctattaa caagagatgg tggt 144

<210> 71
 <211> 144
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: synthetic Env
 US4 common region

<400> 71
 gacaccatca tcctgccctg ccgcatccgc cagatcatca acatgtggca ggaggtgggc 60
 aaggccatgt acgccccccc catccgcggc cagatcaagt gcagcagcaa catcaccggc 120
 ctgctgctga cccgcgacgg cggc 144

<210> 72
 <211> 144
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence: synthetic Env
 SF162 common region

<400> 72
 ggcaccatca ccctgccctg ccgcatcaag cagatcatca accgctggca ggaggtgggc 60
 aaggccatgt acgccccccc catccgcggc cagatccgct gcagcagcaa catcaccggc 120
 ctgctgctga cccgcgacgg cggc 144

<210> 73
 <211> 4766
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:
 gp160.modUS4.gag.modSF2

<400> 73
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 cccgtgtgga aggaggccac caccaccctg ttctgcgcca gcgacgcaa ggcttacaag 180
 gccgaggccc acaacgtgtg ggccaccac gctgctgtgc ccaccgacc caacccccag 240
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gacagctggg	agaagatgcc	cgagggcgag	atcaagaact	gcagcttcaa	catcaccacc	540
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agaattcaga ctcgagcaag tctaga 4766

```

<210> 74

<211> 4689

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

gp160.modSF162.gag.modSF2

<400> 74

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<211> 4472
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<213> Artificial Sequence

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<212> DNA

<213> Artificial Sequence

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agaagcagga gcccatcgac aaggagctgt accccctgac cagcctgcgc agcctgttcc 4560
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```

<210> 77

<211> 1680

<212> DNA

<213> Human immunodeficiency virus

<400> 77

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atggaaaagg aagggaataa ttcaaaaatt gggcctgaaa atccatacaa tactccagta 180
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ataatagagc agttaataaa aaaggaaaag gtctacctgg catgggtacc agcacacaaa 1620
ggaattggag gaaatgaaca agtagataaa ttagtcagtg ctggaatcag gaaagtacta 1680

```

<210> 78

<211> 1865

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: GP1

<400> 78

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gagaagatcc gcctgcgccc cggcggcaag aagaagtaca agctgaagca catcgtgtgg 120
gccagccgag agctggagcg cttcgccgtg aaccccgcc tgctggagac cagcgagggc 180
tgccgccaga tcctgggcca gctgcagccc agcctgcaga ccggcagcga ggagctgcgc 240
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accaaggagg ccctggagaa gatcgaggag gagcagaaca agtccaagaa gaaggcccag 360
caggccgccc ccgcccggg caccggcaac agcagccagg tgagccagaa ctaccccatc 420
gtgcagaacc tgcagggcca gatggtgcac caggccatca gccccgcac cctgaacgcc 480
tgggtgaagg tggtggagga gaaggccttc agccccgagg tgatccccat gttcagcgcc 540
ctgagcgagg gcgccacccc ccaggacctg aacacgatgt tgaacaccgt gggcgccac 600
caggccgcca tgcagatgct gaaggagacc atcaacgagg agggcgccga gtgggaccgc 660
gtgcaccccc tgcagccgg ccccatcgcc cccggccaga tgcgcgagcc ccgcccagc 720
gacatcgccc gcaccaccag caccctgcag gagcagatcg gctggatgac caacaacccc 780
cccatcccc tgggcgagat ctacaagcgg tggatcatcc tgggcctgaa caagatcgtg 840
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gcaacctgct gaccagatc ggctgcacc tgaacttccc catcagcccc atcgagacgg 1800
tgcccgtaga gctgaagccg gggatggacg gcccgaaggt caagcagtgg cccctgtaag 1860
aatcc 1865

<210> 79

<211> 1865

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: GP2

<400> 79

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gccagccgag agctggagcg cttcgccgtg aaccccgcc tgctggagac cagcgagggc 180
tgccgccaga tcctgggcca gctgcagccc agcctgcaga ccggcagcga ggagctgcgc 240
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accaaggagg ccctggagaa gatcgaggag gagcagaaca agtccaagaa gaaggcccag 360
caggccgccc ccgcccggg caccggcaac agcagccagg tgagccagaa ctaccccatc 420
gtgcagaacc tgcagggcca gatggtgcac caggccatca gccccgcac cctgaacgcc 480

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tgggtgaagg tgggtggagga gaaggccttc agccccgagg tgatcccat gttcagcgcc 540
ctgagcgagg gcgccacccc ccaggacctg aacacgatgt tgaacaccgt gggcggccac 600
caggccgcca tgcagatgct gaaggagacc atcaacgagg aggccgcca gtgggaccgc 660
gtgcaccccg tgcacgcccg ccccatcgcc cccggccaga tgcgcgagcc ccgcggcagc 720
gacatcgccg gcaccaccag caccctgcag gagcagatcg gctggatgac caacaacccc 780
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tgcccgtaga gttgaagccg gggatggacg gccccagggt caagcaatgg ccattgtaag 1860
aatte 1865

```

<210> 80

<211> 2305

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

FS(+) .proinact.RTopt.YM

<400> 80

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cagccccacc agaagagagc ttcaggtttg gggaggagaa acaactccc tctcagaagc 180
aggagccgat agacaaggaa ctgtatcctt taacttccct cagatcactc tttggcaacg 240
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acataaagct ataggtacag tattagtagg acctacacct gtcaacataa ttggaagaaa 480
tctgttgacc cagatcggct gcaccttgaa cttccccatc agccctattg agacgggtgc 540
cgtgaagttg aagccgggga tggacggccc caaggtcaag caatggccat tgaccgagga 600
gaagatcaag gccctgggtg agatctgcac cgagatggag aaggagggca agatcagcaa 660
gatcggtccc gagaacccct acaacacccc cgtgttcgcc atcaagaaga aggacagcac 720
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```

<210> 81

<211> 2299

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

FS (+) .proinact.RTopt.YMWM

<400> 81

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cagccccacc	agaagagagc	ttcagggttg	gggaggagaa	aacaactccc	tctcagaagc	180
aggagccgat	agacaaggaa	ctgtatcctt	taacttcctt	cagatcactc	tttggcaacg	240
acctctgct	acaataagga	tcggggggca	actcaaggaa	gcgctgctcg	atacaggagc	300
agatgataca	gtattagaag	aatgaattt	gccaggaaaa	tggaaaccaa	aatgatag	360
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acataaagct	ataggtacag	tattagttagg	acctacacct	gtcaacataa	ttggaagaaa	480
tctgttgacc	cagatcggct	gcaccttgaa	cttccccatc	agccctattg	agacggtgcc	540
cgtgaagttg	aagccgggga	tggacggccc	caaggtcaag	caatggccat	tgaccgagga	600
gaagatcaag	gccctggtgg	agatctgcac	cgagatggag	aaggagggca	agatcagcaa	660
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gcccttcgc	aagcagaacc	ccgcatctgt	gatctaccag	gcccccttgt	acgtgggcag	1080
cgacctggag	atcgccagc	accgaccaa	gatcgaggag	ctgcgccagc	acctgctgcg	1140
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cgtgaacgac	atccagaagc	tggtgggcaa	gctgaactgg	gccagccaga	tctacgccgg	1320
catcaagqqtg	aagcagctgt	gcaagctgct	gcgcggcacc	aaggccctga	ccgaggtgat	1380

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ccccctgacc gaggaggccg agctggagct ggccgagaac cgcgagatcc tgaaggagcc 1440
cgtgcacgag gtgtactacg accccagcaa ggacctggtg gccgagatcc agaagcaggg 1500
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gtacatggac gacctgtacg tgggcagcgg cggccctagg atcgattaaa agcttcccgg 2280
ggctagcacc ggtgaattc                                2299

```

<210> 82

<211> 2306

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

FS (-) .protmod.RTopt.YM

<400> 82

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gcggccgcga aggacaccaa atgaaagatt gcactgagag acaggctaata ttcttccgcg 60
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acagccccac ccgcccgcgag ctgcaggtgt ggggcggcga gaacaacagc ctgagcgagg 180
ccggcgccga ccgcccagggc accgtgagct tcaacttccc ccagatcacc ctgtggcagc 240
gccccctggt gacctcagg atcggcggcc agctcaagga ggcgctgctc gacaccggcg 300
ccgacgacac cgtgctggag gagatgaacc tgcccggcaa gtggaagccc aagatgatcg 360
cggggatcgg gggcttcatc aaggtgcggc agtacgacca gatccccctg gagatctgcg 420
gccacaaggc catcggcacc gtgctggtgg gccccacccc cgtgaacatc atcggccgca 480
acctgctgac ccagatcggc tgcacctga acttccccat cagccccatc gagacggtgc 540
ccgtgaagct gaagccgggg atggacggcc ccaaggtcaa gcagtggccc ctgaccgagg 600
agaagatcaa ggccctggtg gagatctgca ccgagatgga gaaggagggc aagatcagca 660
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ccaagtggcg caagctggtg gacttccgcg agctgaacaa gcgcacccag gacttctggg 780
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agcagggcca gggccagtgg acctaccaga tctaccagga gcccttcaag aacctgaaga 1560
ccggcaagta cgcccgcatg cgcggcgccc acaccaacga cgtgaagcag ctgaccgagg 1620

```



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ccgtgcagaa ggtgagcacc gagagcatcg tgatctgggg caagatcccc aagttcaagc 1680
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ttcccggggc tagcaccggt gaattc 2306

```

<210> 83

<211> 2300

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

FS (-).protmod.RTopt.YMWM

<400> 83

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acagccccac ccgccgcgag ctgcaggtgt gggcgcgcca gaacaacagc ctgagcgagg 180
ccggcgccga ccgccagggc accgtgagct tcaacttccc ccagatcacc ctgtggcagc 240
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ccgagcacac cgtgctggag gagatgaacc tgcccgccaa gtggaagccc aagatgatcg 360
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ccatcgtggg cgccgagacc ttctacgtgg acggcgccgc caaccgcgag accaagctgg 1860

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gcaaggccgg ctacgtgacc gaccggggcc ggcagaaggt ggtgagcatc gccgacacca 1920
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gcgcccggcat ccgcaaggtg ctgttcctga acggcatcga tggcggcatc gtgatctacc 2220
agtacatgga cgacctgtac gtgggcagcg gcggccctag gatcgattaa aagcttcccg 2280
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<210> 84

<211> 2312

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:

FS(-).protmod.RTopt(+)

<400> 84

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agaaggtgta cctggcctgg gtgcccggcc acaagggcat cggcggcaac gagcaggtgg 2160
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<210> 85
 <211> 306
 <212> DNA
 <213> Human immunodeficiency virus

<400> 85
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 gacagtgagg ttcatacaagt ttctctacca aagcaaccgg cttcccagcc ccaaggggac 240
 ccgacaggcc cgaaggaatc gaagaagaag gtggagagag agacagagac agatccagtc 300
 cattag 306

<210> 86
 <211> 101
 <212> PRT
 <213> Human immunodeficiency virus

<400> 86
 Met Glu Pro Val Asp Pro Arg Leu Glu Pro Trp Lys His Pro Gly Ser
 1 5 10 15
 Gln Pro Lys Thr Ala Cys Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe
 20 25 30
 His Cys Gln Val Cys Phe Ile Thr Lys Gly Leu Gly Ile Ser Tyr Gly
 35 40 45
 Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro Pro Asp Ser Glu Val
 50 55 60
 His Gln Val Ser Leu Pro Lys Gln Pro Ala Ser Gln Pro Gln Gly Asp
 65 70 75 80
 Pro Thr Gly Pro Lys Glu Ser Lys Lys Lys Val Glu Arg Glu Thr Glu
 85 90 95
 Thr Asp Pro Val His
 100

<210> 87
 <211> 306
 <212> DNA
 <213> Artificial Sequence
 <220>
 <223> Description of Artificial Sequence: tat.SF162.opt

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aagggcctgg gcatcagcta cggccgcaag aagcgccgcc agcgccgccg cgccccccc 180
gacagcgagg tgcaccaggt gagcctgccc aagcagcccg ccagccagcc ccagggcgac 240
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cactag 306

<210> 88
<211> 306
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
tat.cys22.SF162.opt

<400> 88
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aagggcctgg gcatcagcta cggccgcaag aagcgccgcc agcgccgccg cgccccccc 180
gacagcgagg tgcaccaggt gagcctgccc aagcagcccg ccagccagcc ccagggcgac 240
cccaccggcc ccaaggagag caagaagaag gtggagcgcg agaccgagac cgaccccggtg 300
cactag 306

<210> 89
<211> 168
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
tatamino.SF162.opt

<400> 89
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gcctgcacca actgctactg caagaagtgc tgcttccact gccaggtgtg cttcatcacc 120
aagggcctgg gcatcagcta cggccgcaag aagcgccgcc agcgccgc 168

<210> 90
<211> 102
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: tat cys22
SF162 protein

<400> 90
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1 5 10 15
Gln Pro Lys Thr Ala Gly Thr Asn Cys Tyr Cys Lys Lys Cys Cys Phe
20 25 30

His Cys Gln Val Cys Phe Ile Thr Lys Gly Leu Gly Ile Ser Tyr Gly
 35 40 45
 Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala Pro Pro Asp Ser Glu Val
 50 55 60
 His Gln Val Ser Leu Pro Lys Gln Pro Ala Ser Gln Pro Gln Gly Asp
 65 70 75 80
 Pro Thr Gly Pro Lys Glu Ser Lys Lys Lys Val Glu Arg Glu Thr Glu
 85 90 95
 Thr Asp Pro Val His Glx
 100